Validity and Reproducibility of a Self-Administered Food-Frequency Questionnaire to Assess Isoflavone Intake in a Japanese Population in Comparison with Dietary Records and Blood and Urine Isoflavones1

Seiichiro Yamamoto,2 Tomotaka Sobue, Satoshi Sasaki, Minatsu Kobayashi, Yusuke Arai,* Mariko Uehara,* Herman Adlercreutz, † Shaw Watanabe,* Tosei Takahashi,* Yoji Iitoi,* Yasuhiko Iwase,* Masayuki Akabane* and Shoichiro Tsugane

National Cancer Center Research Institute, Tokyo and Kashiwa, Japan; *Tokyo University of Agriculture, Tokyo, Japan; and †Institute for Preventive Medicine, Nutrition and Cancer, Folkhålsan Research Center and Division of Clinical Chemistry, PB60, 14 University of Helsinki, Finland

ABSTRACT Valid food-frequency questionnaires (FFQ) need to be developed to assess isoflavone intake in investigations of its possible association with the lower incidence of breast and prostate cancer in Asian countries. We investigated the validity and reproducibility of isoflavone (daidzein and genistein) intakes from self-administered semiquantitative FFQ used in the JPHC Study (Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases). We also investigated the number of food items that would be sufficient to ensure validity and reproducibility. We collected FFQ, dietary records (DR), blood and urine samples from 215 subjects among JPHC Study participants, estimated isoflavone intakes from FFQ and DR, and measured serum isoflavone concentration and urine isoflavone excretion. For daidzein, mean intakes estimated from FFQ and DR, serum concentration and urine excretion were 18.3 mg/d, 14.5 mg/d, 119.9 nmol/L and 17.0 μmol/d and for genistein, 31.4 mg/d, 23.4 mg/d, 475.3 nmol/L and 14.2 μmol/d, respectively. Results were similar when analyzed by sex. Spearman correlation coefficients for daidzein of energy-adjusted intakes from FFQ with those from DR, serum concentration and creatinine-adjusted urinary excretion were 0.64, 0.31 and 0.43, respectively. Correlations between two FFQ estimates with a 1-y interval were 0.76. Results were similar for genistein. The shorter version of the FFQ with three items (natto, miso and tofu for miso soup) showed a similar correlation. The original FFQ and the shorter versions have sufficient validity and reproducibility to be used in epidemiologic studies. J. Nutr. 131: 2741–2747, 2001.

KEY WORDS: • Isoflavone • validity • semiquantitative food-frequency questionnaire • dietary record • biomarker • JPHC Study

Asian countries such as Japan have lower incidences of breast and prostate cancer than Western countries. A major reason for the disparity may be the difference in diets, including differences in soybean intake. Soybeans are a major source of isoflavones, one group of phytoestrogens. Many experimental studies have shown anticarcinogenic effects of soy or genistein on hormone-related cancers, which may be related to their estrogenic, antiestrogenic or other effects (1–3). In addition, their anticarcinogenic effects on other cancers such as colon and liver cancer have also been demonstrated in experimental studies. Other possible mechanisms by which soybean isoflavones may be anticarcinogenic include inhibition of protein tyrosine kinases and other enzyme activities, stimulation of sex hormone–binding globulin production, antioxidant effects and inhibition of angiogenesis (1,2,4). Contrary to the results from experimental studies, epidemiologic studies have not provided sufficient evidence of an association between isoflavone intake and cancer (5). Studies to investigate such an association should be conducted in Asian countries because isoflavone intakes vary more widely than in Western countries. Unfortunately, however, an association between isoflavone intake and cancer has not been reported from Asian countries. One reason is that no validated tools exist that can be used in epidemiologic studies to estimate isoflavone intake.

In this study, we developed estimation methods of isoflavone intake using a semiquantitative food-frequency questionnaire (FFQ)3 and evaluated its validity and reproducibility.
This FFQ was originally developed and used in the 5-year follow-up survey of the Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases (JPHC Study), which is an ongoing large-scale cohort study, including approximately 140,000 Japanese subjects (6,7). The present study was conducted as a part of validation studies for the FFQ of the JPHC Study Cohort I (8,9). Estimated isoflavone intakes from FFQ were compared with those from dietary records (DR), serum isoflavone levels and urinary isoflavone excretion to investigate their validity. Reproducibility of the FFQ estimates was also investigated by comparing two FFQ estimates. The association between the biomarkers and DR estimates was also studied. In addition, we investigated how many food items are sufficient to ensure validity and reproducibility.

SUBJECTS AND METHODS

Study subjects were a subsample of the participants in the JPHC Study Cohort I. A total of 247 subjects [122 men and 125 women, 56 from the Ninohe public health center (PHC) area in Iwate Prefecture, 71 from the Yokote PHC area in Akita Prefecture, 60 from the Sakhu PHC area in Nagano Prefecture and 60 from the Ishikawa PHC area in Okinawa Prefecture] volunteered initially. The subjects provided 7-d DR four times (a total of 28 d) in different seasons of the year, i.e., winter (February-March), spring (May-June), summer (August-September) and autumn (November-December) in 1994. In the Ishikawa PHC, with its subtropical climate, 7-d diet records were collected only twice (winter and summer, 1994), because seasonal variations are expected to be small. Mean ages were 55.6 y for men and 55.3 y for women. Mean height, weight and body mass index were 164.5 cm, 65.8 kg and 24.3 kg/m², respectively, for men and 151.1 cm, 53.3 kg, 23.9 kg/m², respectively, for women. The mean intakes of energy, protein, total fat and carbohydrate were 9816.9 kJ/d, 76.2 g/d, 52.9 g/d, 256.9 g/d, respectively, for women. These figures were close to those reported in the National Nutritional Survey of Japan in 1980. In this observational study, study participation and all of the data collection were on a voluntary basis. Oral informed consent was obtained from all of the subjects.

Estimation of isoflavone intake from DR and FFQ. Subjects with complete dietary records (i.e., 28 d for the Ninohe, Yokote and Sakhu areas and 14 d for the Ishikawa area) and the first FFQ were used for the analysis (total 215, including 102 men and 113 women). The isoflavone intakes were estimated using the specifically developed food composition table for isoflavones in Japanese foods (12,13). DR estimates were calculated as the mean intakes over 14 d (Ishikawa) or 28 d (other three areas). FFQ estimates were calculated using the following eight items in the FFQ: mijo (fermented soybean paste) soup, tofu for miso soup, tofu for other dishes, yushi-tofu, freeze-dried tofu, deep-fried tofu, natto (fermented soybeans) and soymilk. For miso soup, the questions on frequency of intakes ranged from almost none to 1–3 times/d, 1–2 times/d, 3–4 times/d, 5–6 times/d, once/d, 2–3 times/d, 4–6 times/d and 7 times/d. The portion sizes were set as follows: 20 g (tofu for mijo soup), 75 g (tofu for other dishes), 150 g (yushi-tofu), 60 g (freeze-dried tofu), 2 g (deep-fried tofu) and 50 g (natto). The categories of relative portion size ranged from small (50% smaller) to medium, and large (50% larger). Because only one frequency (same categories as others) was asked for soymilk, 200 mL was used as the portion size. Isoflavone intake was estimated by the frequency, portion size, relative portion size and isoflavone content in the food composition tables. The relative salt (miso) content (0.75 for less salty, 1 for normal and 1.3 for salty) was also used for the calculation of isoflavone intake from mijo soup.

From the subjects with complete dietary records a second FFQ was obtained and analyzed for 93 men and 109 women to investigate FFQ reproducibility. The validity and reproducibility of the “hypothetical” short versions of the original FFQ (i.e., 8 items) were investigated to assess isoflavone intake. The hypothetical shorter versions were constructed as follows: combinations of 2–7 food items from the original FFQ, and 5 single food items each of which was included in the original FFQ.

Analysis of serum isoflavones. Serum taken in the winter session was used for the study. Among the subjects with complete dietary records, serum was obtained and analyzed for 93 men and 109 women. A sensitive and convenient time-resolved fluoroimmunoassay (TR-FIA) method was used for analysis of serum isoflavones (14) at Helsinki University.

For the recovery calculation, 20 μL of 3H-estradiol glucuronide was added to tubes containing 200 μL of serum. After mixing and equilibrating for 30 min at room temperature, 200 μL of 0.1 mol/L acetate buffer (pH 5.0) containing 200 μL glucuronidase and 200 μL sulfatase was added to the tubes. After mixing using a vortex mixer and incubation overnight at 37°C, 2 mL of diethyl ether was added, and the phytoestrogens were extracted after equilibrating the phases with a vortex mixer. The water phase was frozen in a solid carbon dioxide/ethanol mixture, and the ether phase was transferred into disposable glass tubes. After thawing, the water phase was reextracted with the same amount of ether, and the ether phases were combined and evaporated completely in a 45°C water bath. Then 200 μL of 50 mmol/L Tris-HCl buffer containing 5 μL bovine serum albumin (pH 7.8) (assay buffer) was added to the tubes containing the dry residues; after thorough mixing, 20 μL (in duplicate) of the solution was taken for TR-FIA of each compound. This volume corresponds to 20 μL of the original serum sample. The samples giving a value outside the range of the standard curve were diluted with assay buffer. Another 20 μL of the solution was taken for liquid scintillation counting for determination of recovery. On the basis of these results, the final values were corrected for losses during hydrolysis and extraction.

Standard or hydrolyzed and extracted serum (20 μL) was pipetted
into prewashed goat anti rabbit immunoglobulin G microtitration wells. To each well was added 100 µL of a polyclonal antiserum (dilution of 1:40,000 for daidzein and 1:50,000 for genistein) in assay buffer and 100 µL of europium-labeled daidzein or genistein (dilution 1:40,000 and 1:400,000 for daidzein and genistein, respectively). After incubation and shaking the strips slowly on a DELFIA plate shaker (Wallac, Turku, Finland) at room temperature for 90 min, the strips were washed with a DELFIA plate washer (using the no. 29 T3 program). Enhancement solution (200 µL) was added to each well, and the strips were shaken slowly for an additional 5 min. The enhanced fluorescence was measured in a VICTOR 1420 multilabel counter (Wallac, Turku, Finland). Complete validation of the method has been published (14).

Calculation of the serum isoflavone concentration was done according to the formula: serum isoflavone (nmol/L) = concentration (read) × 1/recovery × dilution factor (nmol/L).

Analysis of urinary isoflavones. The 24-h urine samples taken in the spring session were used for the study. Among the subjects with complete dietary records, urine samples were obtained and analyzed for 33 men and 60 women. Creatinine and isoflavones were measured in the urine samples. Creatinine was analyzed by Jaffe’s procedure. The urinary isoflavones and metabolites were analyzed at Tokyo University of Agriculture using the extraction method of Adlercreutz et al. (15) combined with the modified HPLC method described by Gamache et al. (16). For the recovery calculation, 20 µL of 1H-estradiol glucuronide was added to the tubes containing 1 mL of urine. After mixing and equilibrating for 30 min at room temperature, 0.5 mL of a 0.5 M HCl solution was added to the tubes, and the residue was dissolved in 0.2 mL methanol, and a 20-µL sample was injected onto a HPLC column with diode-array UV detection, scanning from 250 to 400 nm (Beckman Coulter K.K., Tokyo, Japan). Another 20 µL of the solution was taken for liquid scintillation counting for a determination of recovery. Peaks were detected at 254 nm for daidzein and genistein at 258 nm for epsilon and O-DMA. The HPLC column was ODS-80Ts-Qa (150 × 4.6 mm i.d., 3-µm particle size; Tosoh, Tokyo, Japan) with a guard column (TSKguardgel ODS-80Ts, 1.5 × 3.2 mm i.d., 3-µm particle size; Tosoh), and temperature was kept at 25°C using a column oven. HPLC analysis was carried out by linear gradient, from 1.50:5.8:0.2 (methanol/acetone triethylamine/0.2 mol/L acetic acid) to 0.63:0.3:0.17 for 45 min, returning to its initial condition for 5 min. The flow rate was 1.0 mL/min.

Quantification was done by measuring peak areas based on calibration plots of the peak area of standards at various concentrations (from 4 to 160 µmol/L) and corrected for losses during hydrolysis and extraction based on the recovery data. All solvents and chemicals were of HPLC grade or analytical grade.

Intra- and interassay CV for the present method were assessed by repeated measurement of isoflavones in three urine samples with different concentrations. Both intra- and interassay CV were <10% in the three different concentrations.

Calculation of urine isoflavones was done according to the formula: urine isoflavones (µmol/d) = concentration (µmol/L) × 1/recovery × urine volume (L/d).

Statistical analysis. Mean, standard deviation and quartiles of the distribution were calculated to compare the distribution of FFQ estimates, DR estimates, serum levels and urine excretions. To check the normality of the distribution, skewness and kurtosis were calculated. To compare the means, t test was used. The contribution of food items to DR estimates and FFQ estimates was also examined. To investigate the validity of the intake estimates from FFQ, correlation coefficients among FFQ estimates, DR estimates, serum levels and urine excretions were calculated. Correlation coefficients with energy adjustment (intake estimates) and creatinine-adjustment (urine excretion) were also calculated. Energy and creatinine were adjusted by residual methods. Seasonal variation in DR estimates and their correlation with other estimates were also investigated. Reproducibility of FFQ estimates was evaluated by correlation coefficients between two FFQ estimates. All of the correlation coefficients are shown with their 95% confidence interval to evaluate the accuracy of the estimates. Validity and reproducibility of the shorter versions of the original FFQ were also investigated by the analysis described above. All analyses were conducted by sex, but results were pooled when there were no gender differences. Statistical analysis were conducted using SAS Software version 6.12 (SAS Institute, Cary, NC).

RESULTS

The distribution of FFQ estimates, DR estimates, serum levels and urine excretion of isoflavones are shown in Table 1. Skewness and kurtosis showed different patterns among the estimates. Only DR estimates showed approximate normality. Estimated mean intakes were higher as assessed by FFQ than by DR (P < 0.001). Variations in intake were greater in FFQ estimates than in DR estimates in terms of standard deviations, interquartile ranges and skewness.

The contribution of food items to DR and FFQ estimates are shown in Table 2. Food items appearing in the DR, originally categorized by the items in the standard food composition table (14), were categorized into the same items in the FFQ. Only four items (tofu for miso soup, other tofu, miso and natto) contributed >80% of total isoflavone intake in

| TABLE 1 |

<table>
<thead>
<tr>
<th>Isoflavone intake assessed by food-frequency questionnaire (FFQ) and by dietary records (DR), serum isoflavone and urine isoflavone excretion in Japanese subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Daidzein&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intake (FFQ), mg/d</td>
</tr>
<tr>
<td>Intake (DR), mg/d</td>
</tr>
<tr>
<td>Serum, nmol/L</td>
</tr>
<tr>
<td>Urine, µmol/d</td>
</tr>
<tr>
<td>Genistein&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intake (FFQ), mg/d</td>
</tr>
<tr>
<td>Intake (DR), mg/d</td>
</tr>
<tr>
<td>Serum, nmol/L</td>
</tr>
<tr>
<td>Urine, µmol/d</td>
</tr>
</tbody>
</table>

<sup>1</sup> Q1, 25th percentile; Q2, median; Q3, 75th percentile.  
<sup>2</sup> Molecular weight of daidzein is 254.24.  
<sup>3</sup> Molecular weight of genistein is 270.24.
The results did not change substantially when Pearson’s correlation coefficients were used instead of Spearman’s.

The seasonal variation in DR estimates and their correlation with other estimates are shown in Table 4. Seasonal variation was relatively small in terms of variance ratio of intra- to inter-individual variation i.e., 1.11 for daidzein and 1.06 for genistein. Season specific DR estimates showed more consistent correlation with FFQ estimates, which were obtained in winter, than with biomarkers. Among the correlations with season specific DR estimates, serum levels and urine excretion showed the highest correlations in winter and spring, respectively, in which the samples were taken. With the DR estimates in other seasons, serum levels showed a relatively high correlation but urine excretion showed an unstable correlation.

Results for the validity and reproducibility of the shorter versions of the original FFQ are shown in Table 5 for daidzein. Validity was investigated by Spearman’s correlation coefficients between the intake estimates from the shorter versions and the original FFQ estimates (8 items included), DR estimates, serum isoflavones and urine excretion. Reproducibility was investigated by Spearman’s correlation coefficients between the shorter versions of the two original FFQ. The shorter versions were constructed by gradually omitting those items that contributed least to isoflavone intake in the FFQ estimates (Table 3). Correlation coefficients for the shorter versions with more than one item were not different from those for the original FFQ except for the low correlation between the two-item FFQ and urine excretion. Furthermore, even higher correlation coefficients were observed for FFQ with five items. Among the food items, natto, miso and tofu in miso soup had high correlations with other estimates, whereas yushi-tofu and soymilk had low correlations. The shorter versions with more than one item and individual items except tofu (others) and soymilk showed high reproducibility. The results for genistein were similar (not shown).

### DISCUSSION

In the validation study of FFQ, correlations with the gold standard (validity) or between repeated measures (reproducibility), may seem low to those observed in the validity and reproducibility of laboratory measurements made under highly controlled conditions. One reason for these low correlations arises from the measurement error included in the gold standard (DR). Nevertheless, correlation coefficients on the order of 0.5–0.7 are comparable to the validities of other epidemiological methods of exposure assessment.

### TABLE 2

**Contribution of food items to isoflavone intake estimated by dietary records (DR) and food-frequency questionnaire (FFQ) in Japanese subjects**

<table>
<thead>
<tr>
<th>Food item</th>
<th>DR Intake</th>
<th>DR Contribution</th>
<th>FFQ Intake</th>
<th>FFQ Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/d</td>
<td>%</td>
<td>mg/d</td>
<td>%</td>
</tr>
<tr>
<td>Daidzein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natto</td>
<td>2.40</td>
<td>16.5</td>
<td>6.21</td>
<td>33.9</td>
</tr>
<tr>
<td>Miso</td>
<td>3.84</td>
<td>26.4</td>
<td>4.54</td>
<td>24.8</td>
</tr>
<tr>
<td>Tofu</td>
<td>6.06</td>
<td>41.8</td>
<td>5.58</td>
<td>30.5</td>
</tr>
<tr>
<td>(Miso soup)</td>
<td>—</td>
<td>—</td>
<td>(2.86)</td>
<td>(15.7)</td>
</tr>
<tr>
<td>(Others)</td>
<td>—</td>
<td>—</td>
<td>(2.71)</td>
<td>(14.8)</td>
</tr>
<tr>
<td>Freeze-dried tofu</td>
<td>0.17</td>
<td>1.2</td>
<td>1.42</td>
<td>7.8</td>
</tr>
<tr>
<td>Yushi-tofu</td>
<td>0.91</td>
<td>6.2</td>
<td>0.26</td>
<td>1.4</td>
</tr>
<tr>
<td>Soymilk</td>
<td>0.00</td>
<td>0.0</td>
<td>0.23</td>
<td>1.2</td>
</tr>
<tr>
<td>Deep-fried tofu</td>
<td>0.39</td>
<td>2.7</td>
<td>0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>0.75</td>
<td>5.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>14.52</td>
<td>100.0</td>
<td>18.29</td>
<td>100.0</td>
</tr>
<tr>
<td>Genistein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natto</td>
<td>3.98</td>
<td>17.0</td>
<td>10.29</td>
<td>32.8</td>
</tr>
<tr>
<td>Miso</td>
<td>5.30</td>
<td>22.7</td>
<td>6.26</td>
<td>20.0</td>
</tr>
<tr>
<td>Tofu</td>
<td>9.87</td>
<td>42.2</td>
<td>9.04</td>
<td>28.8</td>
</tr>
<tr>
<td>(Miso soup)</td>
<td>—</td>
<td>—</td>
<td>(4.64)</td>
<td>(14.8)</td>
</tr>
<tr>
<td>(Others)</td>
<td>—</td>
<td>—</td>
<td>(4.40)</td>
<td>(14.0)</td>
</tr>
<tr>
<td>Freeze-dried tofu</td>
<td>0.56</td>
<td>2.4</td>
<td>4.69</td>
<td>14.9</td>
</tr>
<tr>
<td>Yushi-tofu</td>
<td>1.91</td>
<td>8.2</td>
<td>0.55</td>
<td>1.8</td>
</tr>
<tr>
<td>Soymilk</td>
<td>0.01</td>
<td>0.0</td>
<td>0.46</td>
<td>1.5</td>
</tr>
<tr>
<td>Deep-fried tofu</td>
<td>0.78</td>
<td>3.3</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>0.96</td>
<td>4.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>23.36</td>
<td>100.0</td>
<td>31.39</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### TABLE 3

**Correlation matrix of isoflavone intake from food-frequency questionnaire (FFQ) and dietary records (DR), serum isoflavones and urine isoflavone excretion in Japanese subjects**

<table>
<thead>
<tr>
<th></th>
<th>Daidzein</th>
<th>Genistein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td>FFQ vs. DR</td>
<td>0.57 (0.47–0.67)</td>
<td>0.60 (0.50–0.69)</td>
</tr>
<tr>
<td>FFQ vs. Serum</td>
<td>0.31 (0.18–0.44)</td>
<td>0.26 (0.13–0.39)</td>
</tr>
<tr>
<td>FFQ vs. Urine</td>
<td>0.29 (0.09–0.49)</td>
<td>0.40 (0.21–0.60)</td>
</tr>
<tr>
<td>DR vs. Serum</td>
<td>0.39 (0.27–0.51)</td>
<td>0.37 (0.25–0.49)</td>
</tr>
<tr>
<td>DR vs. Urine</td>
<td>0.43 (0.25–0.60)</td>
<td>0.48 (0.31–0.64)</td>
</tr>
<tr>
<td>Serum vs. Urine</td>
<td>0.23 (0.03–0.43)</td>
<td>0.22 (0.01–0.42)</td>
</tr>
</tbody>
</table>

1 95% confidence intervals of Spearman’s correlation coefficients.
2 Adjusted correlation coefficient by total energy for FFQ and DR and creatinine for urine.
Seasonal variation of isoflavone intake estimates from dietary records (DR) and their correlation coefficients with isoflavone intake estimates from food-frequency questionnaire (FFQ), serum isoflavone levels and urine isoflavone excretion in Japanese subjects

<table>
<thead>
<tr>
<th>Natto</th>
<th>Daizein</th>
<th>Genistein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>0.98 (0.96–0.99)</td>
<td>0.98 (0.98–0.99)</td>
</tr>
<tr>
<td>Spring</td>
<td>0.98 (0.97–1.00)</td>
<td>0.97 (0.96–0.99)</td>
</tr>
<tr>
<td>Summer</td>
<td>0.95 (0.94–0.96)</td>
<td>0.92 (0.91–0.94)</td>
</tr>
<tr>
<td>Autumn</td>
<td>0.92 (0.91–0.94)</td>
<td>0.89 (0.88–0.91)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.96 (0.95–0.97)</td>
<td>0.93 (0.92–0.95)</td>
</tr>
</tbody>
</table>

1 Number of subjects used in calculation of Spearman's correlation coefficients in winter, spring, summer and autumn.
2 95% confidence intervals of Spearman's correlation coefficients.

TABLE 5
Correlation coefficients between shorter versions of food-frequency questionnaire (FFQ) and dietary records (DR) for daidzein, serum concentration and urine excretion of daidzein in Japanese subjects

<table>
<thead>
<tr>
<th>Intake (FFQ)</th>
<th>Intake (DR)</th>
<th>Serum</th>
<th>Urine</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 8 items</td>
<td>1.00 (1.00–1.00)</td>
<td>0.57 (0.47–0.67)</td>
<td>0.31 (0.18–0.44)</td>
<td>0.29 (0.09–0.49)</td>
</tr>
<tr>
<td>7 items</td>
<td>0.99 (0.99–1.00)</td>
<td>0.57 (0.47–0.66)</td>
<td>0.31 (0.18–0.44)</td>
<td>0.29 (0.09–0.49)</td>
</tr>
<tr>
<td>6 items</td>
<td>0.98 (0.98–0.99)</td>
<td>0.58 (0.48–0.67)</td>
<td>0.32 (0.19–0.45)</td>
<td>0.28 (0.08–0.49)</td>
</tr>
<tr>
<td>5 items</td>
<td>0.99 (0.99–1.00)</td>
<td>0.59 (0.50–0.69)</td>
<td>0.32 (0.19–0.45)</td>
<td>0.29 (0.09–0.49)</td>
</tr>
<tr>
<td>4 items</td>
<td>0.98 (0.98–0.99)</td>
<td>0.58 (0.49–0.68)</td>
<td>0.31 (0.18–0.45)</td>
<td>0.32 (0.12–0.52)</td>
</tr>
<tr>
<td>3 items</td>
<td>0.94 (0.91–0.96)</td>
<td>0.53 (0.43–0.64)</td>
<td>0.28 (0.15–0.41)</td>
<td>0.35 (0.15–0.55)</td>
</tr>
<tr>
<td>2 items</td>
<td>0.88 (0.84–0.93)</td>
<td>0.52 (0.41–0.63)</td>
<td>0.27 (0.13–0.40)</td>
<td>0.12 (0.01–0.34)</td>
</tr>
<tr>
<td>Natto</td>
<td>0.83 (0.78–0.88)</td>
<td>0.48 (0.37–0.60)</td>
<td>0.23 (0.10–0.36)</td>
<td>0.17 (0.05–0.4)</td>
</tr>
<tr>
<td>Miso</td>
<td>0.57 (0.47–0.67)</td>
<td>0.44 (0.32–0.56)</td>
<td>0.21 (0.07–0.35)</td>
<td>0.07 (0.29–0.14)</td>
</tr>
<tr>
<td>Tofu (miso soup)</td>
<td>0.62 (0.53–0.71)</td>
<td>0.41 (0.29–0.52)</td>
<td>0.19 (0.07–0.32)</td>
<td>0.21 (0.01–0.41)</td>
</tr>
<tr>
<td>Tofu (others)</td>
<td>0.55 (0.44–0.66)</td>
<td>0.25 (0.12–0.38)</td>
<td>0.14 (0.00–0.27)</td>
<td>0.24 (0.05–0.43)</td>
</tr>
<tr>
<td>Freeze-dried tofu</td>
<td>0.47 (0.36–0.53)</td>
<td>0.31 (0.18–0.43)</td>
<td>0.16 (0.02–0.29)</td>
<td>0.16 (0.07–0.37)</td>
</tr>
<tr>
<td>Yushi-tofu</td>
<td>−0.26 (−0.39 to −0.13)</td>
<td>−0.36 (−0.48 to −0.23)</td>
<td>−0.13 (−0.27–0.11)</td>
<td>−0.07 (−0.30–0.16)</td>
</tr>
<tr>
<td>Soy milk</td>
<td>0.19 (0.09–0.28)</td>
<td>−0.10 (−0.23–0.02)</td>
<td>−0.01 (−0.14–0.12)</td>
<td>0.04 (−0.18–0.27)</td>
</tr>
<tr>
<td>Deep-fried tofu</td>
<td>0.50 (0.40–0.60)</td>
<td>0.43 (0.32–0.54)</td>
<td>0.18 (0.05–0.31)</td>
<td>0.09 (−0.11–0.29)</td>
</tr>
</tbody>
</table>

1 Shorter versions of FFQ includes items as follows: all 8 items (all items in original FFQ), 7 items (all items except deep-fried tofu and soy milk), 6 items (all items except deep-fried tofu and soy milk), 5 items (natto, miso, tofu for miso soup, tofu for others, and freeze-dried tofu), 4 items (natto, miso, tofu for miso soup, and tofu for others), 3 items (natto, miso, and tofu for miso soup), 2 items (natto and miso).
2 95% confidence intervals of Spearman’s correlation coefficients.
soup) has sufficient validity and reproducibility and the one-
item FFQ such as natto and miso may be sufficient. Our
 correlations among FFQ, DR and urine excretion were similar
to those in Japanese in the United States assessed using the
Block FFQ, which was modified for Japanese by the addition of
58 items (18).

These isoflavone intakes, blood concentrations and urine excretions were comparable to those cited in other reports of
Japanese subjects and higher than those for Western populations.
Daidzein intake estimates of our subjects were 7 times higher than those of Chinese in Singapore, and 700 times higher than for U.S. Caucasians (13,18–21). A similar, al-
though less striking discrepancy was observed for biomarkers.
Blood daidzein concentrations in our subjects were 15 times higher than those in Finnish omnivorous subjects and 3 times higher than those in Finnish vegetarians (14,22–24). Urine
daidezine excretion in our subjects was 50 times higher than those in Finnish omnivorous subjects and 3 times higher than those in American macrobiotics (22,25–28).

Correlation coefficients of energy/creatinine-adjusted in-
take were higher than those of nonadjusted intakes among
FFQ vs. DR and FFQ vs. urine. These three estimates (FFQ,
DR and urine) have the dimension of l/d and are influenced
by body size. Energy/creatinine adjustment can reduce the
influence of body size, resulting in a possible increase in the
correlation between adjusted estimates. In contrast, not only
did correlation coefficients with serum levels not increase after
energy/creatinine adjustment, some of them actually decreased
after the adjustment. Such decreases may be due to errors in
energy intake estimates.

FFQ estimates tended to be larger than the DR estimates
although the correlation was high. This overestimation was
largely due to the overestimation of isoflavone intake from
natto (Table 2). Although the contribution of natto was
16.5% in DR daidzein estimates, the corresponding figure was
33.9% in FFQ estimates. Because correlations between natto and other estimates were high (Table 5), the natto portion size
used may have been inappropriate. Although the natto por-
tion size was shown as 50 g in the FFQ, 20 g may be a more
appropriate amount to prevent overestimation of FFQ esti-
mates.

Our study subjects were a subsample of the JPHC Study,
which took place in a Japanese population. However, the
subjects were not necessarily representative of all of the JPHC
Study subjects because they were volunteers and possibly more
health conscious than other subjects. This might suggest that
the correlations of FFQ estimates and others observed in this
study were slightly higher than in the general population.

Another limitation of the study was that a single urine and
blood sample was used, although we collected two urine and
blood samples for each subject. This may lead to the low
 correlation between FFQ and DR intake estimates and biomar-
kers because the single measurement has larger measurement
error than the means over repeated samples. However, corre-
lations between FFQ and DR intake estimates and single
biomarker measurements were sufficiently high to be used in
epidemiologic studies.

FFQ estimates correlated consistently with four season-
specific DR estimates. Serum levels also correlated consistently with four season-specific DR estimates although correlations were lower than with FFQ estimates. Urine isoflavones in
samples collected in the spring session had a higher association
with DR estimat es in the spring session compared with FFQ
estimates and serum levels, but the lowest association with the
DR estimates in other seasons. This suggests, in terms of
estimating intakes, that FFQ are the most appropriate measure
for long-term isoflavone intake, serum levels are the second
best, and that urine excretion levels, although not appropriate
for long-term may be the most appropriate measure for esti-
mat ing short-term intakes. In experimental studies, the peak
concentrations of daidzein and genistein in human plasma are
generally seen 4–8 h after the ingestion of glycosides (29–32).
Most of the daidzein and genistein in urine reaches a peak after
8–12 h and is excreted within 48 h after ingestion (30–33).
There is little difference in the apparent peak time of isofla-
vones in biological fluids among races, although differences are
observed in absolute levels due to individual variations in
bioavailability (30,31,34,35).

The correlations between the intake estimates and biomar-
kers observed for daidzein were similar to those for genistein.
This is due in part to the fact that the ratio of genistein to
daidezine in food composition tables did not vary greatly (one-
to-two fold with few exceptions) among food items.

This study showed the high validity and reproducibility of
a FFQ to assess isoflavones intake in a Japanese population
using ≤8 items. This suggests two possibilities. Isoflavone intake can be estimated by a simpler FFQ with fewer items in
an Asian population. This means that it may be possible to
investigate the association between isoflavone intake and can-
cer in cohort studies, even if the study has been conducted in
the past without validation of isoflavone intake. Another pos-
sibility is to obtain improved intake estimates by changing
the portion size such as we did with natto in our FFQ. This
may lead to the development of estimation methods by FFQ
that can circumvent the risk of overestimation. In that case
recommended intakes of isoflavones can be proposed using
FFQ data if isoflavone intake proves to be effective in prevent-
ing cancer.

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