Dietary Soy Isoflavones and Bone Mineral Density: Results from the Study of Women’s Health Across the Nation

Gail A. Greendale,1 Gordon FitzGerald,2 Mei-Hua Huang,1 Barbara Sternfeld,3 Ellen Gold,4 Teresa Seeman,1 Sherry Sherman,5 and MaryFran Sowers6

Isoflavones are naturally occurring selective estrogen receptor modulators, with potential bone protective effects. To study the relation between soy isofoxavone intake and bone mineral density (BMD), the authors analyzed baseline data from the Study of Women’s Health Across the Nation, a US community-based cohort study of women aged 42–52 years. Their 1996–1997 analysis included African-American (n = 497), Caucasian (n = 1,003), Chinese (n = 200), and Japanese (n = 227) participants. Genistein and daidzein intakes were highly correlated (r = 0.96); therefore, analyses were conducted by using genistein. Median intakes of genistein (measured in micrograms/day) by African Americans and Caucasians were too low to pursue relational analyses further. For Chinese and Japanese women, median genistein intakes were 3,511 and 7,151 µg/day, respectively. Ethnic-specific, linear models were used to predict BMD as a function of energy-adjusted tertile of intake, controlled for relevant covariates. For Chinese women, no association between genistein and BMD was found. Premenopausal, but not perimenopausal, Japanese women whose intakes were greater had higher spine and femoral neck BMD. Adjusted mean spinal BMD of those in the highest tertile of intake was 7.7% greater than that of women in the lowest tertile (p = 0.02); femoral neck BMD was 12% greater in the highest versus the lowest tertile (p < 0.0001). Am J Epidemiol 2002;155:746–54.

bone density; cohort studies; diet; genistein; isoflavones; menopause; soybeans; women

Japanese and Chinese women are about half as likely as Caucasian women to experience a hip fracture (1, 2). Understanding the reasons for this striking ethnic difference in osteoporosis occurrence could lead to new strategies to prevent or treat this condition. Ecologic studies point to a high-soy diet as one attractive explanation for the observed lower fracture rate among Asian compared with Caucasian populations (3, 4).

The phytoestrogen components of soybeans and soybean products may account for a bone protective effect of these foods. Phytoestrogens, heterocyclic phenols structurally analogous to human estrogens, bind to estrogen receptors alpha and beta and stimulate transcriptional activity (5–7).

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Abbreviations: BMD, bone mineral density; FFQ, food frequency questionnaire; SWAN, Study of Women’s Health Across the Nation.

1 Division of Geriatrics, School of Medicine, University of California, Los Angeles, Los Angeles, CA.
2 New England Research Institute, Watertown, MA.
3 Division of Research, Kaiser Permanente, Oakland, CA.
4 Department of Epidemiology and Preventive Medicine, School of Medicine, University of California, Davis, Davis, CA.
5 National Institute on Aging, National Institutes of Health, Bethesda, MD.
6 Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI.

Correspondence to Dr. Gail A. Greendale, Division of Geriatrics, School of Medicine, University of California, Los Angeles, 10945 Le Conte Avenue, Suite 2339, Los Angeles, CA 90095-1687 (e-mail: ggreenda@mednet.ucla.edu).
dietary isoflavone intake and BMD? Does this relation vary by race/ethnicity or by menopausal status?

MATERIALS AND METHODS

Study sample

SWAN is a multisite, longitudinal cohort study of midlife in a community-based sample of 3,302 women. Participants were menstruating at baseline and were members of five ethnic groups: African American (n = 935), Caucasian (n = 1,550), Chinese (n = 250), Hispanic (n = 286), and Japanese (n = 281). Eligibility criteria for entry into the SWAN longitudinal cohort were as follows: age 42–52 years, no surgical removal of the uterus and/or both ovaries, not currently using hormones that affect the ovaries, having at least one menses in the 3 months prior to screening, and self-identification as one of the five eligible ethnic groups.

Cohort recruitment and enrollment have been described in detail previously (17). In brief, participants were enrolled at seven clinical sites in the following geographic areas of the United States: Boston, Massachusetts; Chicago, Illinois; Detroit, Michigan; Los Angeles, California; Newark, New Jersey; Oakland, California; and Pittsburgh, Pennsylvania. All seven SWAN clinical sites enrolled Caucasians; the Boston, Chicago, Detroit, and Pittsburgh sites enrolled African Americans; and the remaining three sites enrolled Japanese, Hispanic, and Chinese women, respectively. The Chicago and Newark sites did not perform BMD testing, leaving a potential maximum of 2,413 participants for BMD analyses.

Of the women at the five SWAN BMD testing sites, 82 did not have a baseline measurement of lumbar spine BMD and 54 had technically unsatisfactory baseline scans of spine BMD, resulting in 2,277 participants for whom lumbar spine BMD data were usable (94.4 percent of participants at the five BMD sites). A baseline hip BMD measurement was lacking for 81 women, and three had technically unsatisfactory baseline hip BMD measurements, resulting in 2,329 women at BMD sites with usable data on hip BMD (96.5 percent of women at the BMD sites). Because a few women had some health conditions that affect BMD, we excluded those with self-reported anorexia or bulimia (n = 30 (spine) and n = 32 (hip)), hypercalcemia (n = 13 in each sample), tamoxifen use (n = 4 in each sample), medroxyprogesterone acetate use (n = 21 (spine) and n = 22 (hip)), and corticosteroid use (n = 48 (spine) and n = 50 (hip)). Dietary data quality control exclusions were as follows: recorded intakes of less than four or more than 17 solid foods per day, omission of more than 10 foods when responding to the FFQ, and estimated kilocalorie intakes of less than 500 or more than 5,000 daily.

Standardized, self-report questionnaires were used to assess the covariates of age (years, continuous), current smoking (yes/no) (19), place of birth (US or non-US location), duration of residence in the United States (years), physical activity (summary score of active living, home, and recreational physical activity) (20), history of hyperthyroidism (yes/no), ever use (yes/no) of any estrogen or estrogen/progestin pill or patch (including oral contraceptives or postmenopausal hormone therapy), and mensturally defined menopause status (premenopausal (regular menses), early perimenopausal (menses within the prior 3 months but less predictable)). Intakes of calcium (milligrams/day), protein (kilocalories/day), and alcohol (kilocalories/day) were estimated by using the modified FFQ (18).

Data analysis

First, dietary genistein and daidzein intakes were calculated for each of the four ethnic groups. Among African-American and Caucasian women, the amount of these isoflavones was estimated by using the modified FFQ. Dietary genistein and daidzein intakes were calculated for each of the four ethnic groups. Among African-American and Caucasian women, the amount of these isoflavones was estimated by using the modified FFQ.
isoﬂavones consumed was very low (table 1). Thus, we did
not continue to evaluate the relation between genistein and
BMD for these two ethnic groups. For Chinese and Japanese
women, crude and adjusted mean BMD values were com-
pared by ethnic-speciﬁc tertile of genistein intake. Because
consumption of most nutrients is positively related to total
energy intake, it is necessary to adjust for total energy.
Therefore, our relational analyses used energy-adjusted
genistein as the primary exposure variable (21). Energy-
adjusted genistein was calculated separately for Chinese and
Japanese women by using a linear regression model with
total energy intake as the independent variable and genistein
intake as the dependent variable. Residuals from these
regressions were then used as the genistein exposure vari-
able in models relating genistein intake to BMD. This resid-
ual technique is considered the optimal method of energy
adjustment (21). Analysis of variance (unadjusted) and
analysis of covariance (adjusted) models were used to assess
the relation between genistein and BMD. Adjusted mean
BMD values were controlled for age, current smoking,
physical activity, dietary calcium, dietary alcohol, dietary
protein, height, weight, and menopause status. The follow-
ing were also considered as ﬁnal model covariates and were
so used if statistically signiﬁcant: duration of residence in
the United States, self-reported ever use of estrogen or estro-
gen/progestin (largely the oral contraceptive pill), self-
report of over- or underactive thyroid, and self-reported use
of thyroid pills. Interactions between menopause status and
so used if statistically signiﬁcant.
Ethnic-speciﬁc models disclosed apparent differences in
the relation between genistein and BMD in Japanese and
Chinese participants. These differences could have been due
to differences in genistein dose and/or ethnic differences in
response to genistein. To explore these possibilities, we cre-
ated a general linear model that included both Japanese and
Chinese women whose genistein intakes were 800–30,000
µg per day (about 85 percent of each of these two groups).
This model included energy-adjusted genistein as a continu-
ous variable as well as the same covariates used in the
ethnic-speciﬁc models, and it also added a genistein-by-
ethnicity interaction term. All statistical analyses were per-
formed by using version 6.12 of the Statistical Analysis
System (22).

RESULTS
Ethnic-speciﬁc intakes of dietary genistein, the
isoﬂavone used as the primary exposure variable in this
analysis, are summarized in table 1. Approximately 31 per-
cent of Caucasian women and 45 percent of African-
American women recorded no genistein consumption.
When we considered only those Caucasian and African-
American women who reported eating some genistein-
containing foods, estimated genistein intake values
remained very low: median values were 14 µg for
Caucasians and 4 µg for African Americans. In contrast,
only one Japanese and one Chinese woman had genistein-
free diets. Although more than 99.5 percent of Japanese and
Chinese women reported some intake of genistein, the
distribution of this variable for these participants was moder-
ately skewed. To facilitate comparison of our results with
the published literature, which often considers the effects of
genistein and daidzein in combination, combined intakes of
genistein and daidzein are also presented in table 1. Because
reported consumption of genistein was absent to very low in

<table>
<thead>
<tr>
<th>Genistein and daidzein intakes (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein and daidzein intakes (µg/day)</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td>Genistein</td>
</tr>
<tr>
<td>Entire sample</td>
</tr>
<tr>
<td>Nonzero intakes</td>
</tr>
<tr>
<td>Daidzein</td>
</tr>
<tr>
<td>Entire sample</td>
</tr>
<tr>
<td>Nonzero intakes</td>
</tr>
<tr>
<td>Sum of genistein and daidzein</td>
</tr>
<tr>
<td>Entire sample</td>
</tr>
<tr>
<td>Nonzero intakes</td>
</tr>
</tbody>
</table>

*IQ, interquartile; SD, standard deviation.
† Entire sample sizes were 1,003, 497, 227, and 200 for Caucasian, African-American, Japanese, and Chinese, women, respectively. Values are based on the lumbar spine sample; values for the hip sample did not differ substantively.
‡ Nonzero intake sample sizes were 695, 274, 226, and 199 for Caucasian, African-American, Japanese, and Chinese women, respectively.
§ Interquartile range.
Caucasian and African-American SWAN participants, further analysis of the association between genistein and BMD was not pursued for these women.

Japanese and Chinese women were similar in age, calcium intake, and hormone use but differed according to several other relevant characteristics (table 2). Table 2 presents data for Japanese pre- and perimenopausal women separately, because the relational analyses of genistein to BMD, described subsequently, found different results according to menstrual status of these women.

Genistein consumption was substantially higher in Japanese compared with Chinese women (table 3). The

### TABLE 2. Means and frequencies of selected characteristics of Japanese and Chinese women study participants,* Study of Women's Health Across the Nation, United States, 1996–1997

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Japanese (n = 227)</th>
<th>Chinese (n = 200)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premenopausal</td>
<td>Early perimenopausal‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 138)</td>
<td>(n = 89)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.4 (2.57)</td>
<td>46.5 (2.63)</td>
<td>0.17</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.9 (5.03)</td>
<td>158.0 (5.97)</td>
<td>0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.9 (8.29)</td>
<td>58.4 (10.8)</td>
<td>0.10</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>636.6 (334.6)</td>
<td>677.3 (357.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Alcohol intake (kcal/day)</td>
<td>34.7 (76.2)</td>
<td>10.6 (33.3)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.03 (0.12)</td>
<td>1.04 (0.12)</td>
<td>0.03</td>
</tr>
<tr>
<td>Femoral neck BMD (g/cm²)</td>
<td>0.78 (0.09)</td>
<td>0.78 (0.10)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Total hip BMD (g/cm²)</td>
<td>0.90 (0.11)</td>
<td>0.86 (0.10)</td>
<td>0.0056</td>
</tr>
<tr>
<td>Physical activity¶ (score)</td>
<td>7.85 (1.61)</td>
<td>7.25 (1.63)</td>
<td>0.0004</td>
</tr>
<tr>
<td>No. of years since moving to the United States</td>
<td>31.7 (15.9)</td>
<td>28.2 (14.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Born in the United States</td>
<td>48.5</td>
<td>30.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>10.9</td>
<td>2.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Ever use of estrogen/progestagen#</td>
<td>58.1</td>
<td>65.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Former hyperthyroidism</td>
<td>2.3</td>
<td>6.2</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Sample sizes are for the lumbar spine sample. Sample sizes for the hip sample were 228 (Japanese) and 203 (Chinese). Values for Japanese premenopausal and perimenopausal women are shown separately because relational analyses of genistein and bone mineral density (BMD) differed by menstrual status for Japanese but not Chinese women (refer to the text). Values (other than BMD) are based on the lumbar spine sample; values for covariates in the hip sample did not differ substantively.

† For chi-square tests of categorical variables and analysis of covariance for continuous variables.

‡ Early perimenopausal defined by self-report of menstruating in the past 3 months but having less-predictable menstrual cycles.

§ SD, standard deviation.

¶ Summary score of active daily living, home physical activity, and recreational physical activity; refer to the Materials and Methods section of the text for details.

# Ever use of any estrogen or progestagen (almost entirely oral contraceptives).

### TABLE 3. Tertile distributions of dietary genistein intake among Japanese and Chinese women,* Study of Women's Health Across the Nation, United States, 1996–1997

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Range</th>
<th>Japanese Mean (SD)</th>
<th>Median of tertile</th>
<th>IQ† range</th>
<th>Chinese Mean (SD)</th>
<th>Median of tertile</th>
<th>IQ range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–4,091</td>
<td>2,118 (1,367)</td>
<td>2,235</td>
<td>2,539</td>
<td>0–1,582</td>
<td>821 (470)</td>
<td>930</td>
</tr>
<tr>
<td>2</td>
<td>4,259–12,374</td>
<td>7,715 (2,559)</td>
<td>7,151</td>
<td>4,050</td>
<td>1,591–5,630</td>
<td>3,466 (1,278)</td>
<td>3,511</td>
</tr>
<tr>
<td>3</td>
<td>12,537–47,714</td>
<td>22,586 (8,918)</td>
<td>20,522</td>
<td>13,104</td>
<td>5,634–37,700</td>
<td>13,040 (7,154)</td>
<td>10,537</td>
</tr>
</tbody>
</table>

* Tertiles contain 76, 75, and 76 Japanese and 67, 66, and 67 Chinese women, respectively. Values are based on the spine sample. Values for genistein did not differ between the spine and hip samples. Genistein values were not energy adjusted.

† SD, standard deviation; IQ, interquartile.
median value of each ethnic-specific tertile of genistein intake was approximately two times higher in the former compared with the latter group \( (p = 0.0001, \text{Wilcoxon rank-sum test}) \). Mean values of genistein intake were also statistically significantly greater for Japanese than for Chinese women \((p < 0.0001)\). Tertile cutpoints for genistein intake did not differ substantively between pre- and perimenopausal Japanese women (data not shown).

Table 4 illustrates that energy-adjusted genistein consumption was not associated with BMD among Chinese women. Multiply-adjusted mean lumbar spine BMD values were similar in each tertile of genistein intake. Likewise, adjusted femoral neck BMD values did not vary by genistein intake. Finally, for Chinese women, the effect of genistein on BMD did not differ in pre- compared with perimenopausal women: the \( p \) values for the test of interaction between menopause status and genistein were 0.23 at the spine and 0.88 at the femoral neck. Results were similar for the total hip BMD site (data not shown).

For Japanese participants, the association between BMD and genistein depended on menopause status. The \( p \) values for the interaction between menopause status and genistein were 0.083 at the spine and 0.006 at the femoral neck. Premenopausal, but not perimenopausal, Japanese women whose genistein intakes were greater had higher BMD values at the spine and the femoral neck (table 4). Pairwise comparisons revealed that the adjusted mean spinal BMD of the women in the highest tertile of genistein intake was 1.065 g/cm\(^2\), about 7.7 percent higher than the mean BMD of women in the lowest tertile of genistein intake \((p = 0.02)\). Among premenopausal Japanese women, at the femoral neck, there was a striking dose-response relation between dietary genistein and BMD; the adjusted mean BMD of women in the highest tertile of genistein consumption was 12 percent greater than that of participants in the lowest tertile. At the femoral neck, among premenopausal Japanese women, pairwise comparisons of adjusted mean BMD values by tertile of genistein were as follows: third versus first, \( p < 0.0001\); third versus second, \( p = 0.17\); and second versus first, \( p = 0.001\). There were no statistically significant associations between genistein consumption and spine or femoral neck BMD for Japanese perimenopausal women. Results for total hip BMD did not differ substantively from those for the femoral neck (data not shown).

The relation between BMD and genistein intake differed for Chinese and Japanese participants: we found no association for Chinese women but a substantial, positive association for Japanese women but a substantial, positive association for Chinese and Japanese participants: we found no association for Chinese and Japanese participants: we found no association for Chinese and Japanese participants: we found no association for Chinese and Japanese participants: we found no association for Chinese women, Study of Women’s Health Across the Nation, United States, 1996–1997

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Gender</th>
<th>Tertile</th>
<th>Mean BMD (g/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese</td>
<td>Premenopausal</td>
<td>1</td>
<td>0.989</td>
</tr>
<tr>
<td>Japanese</td>
<td>Premenopausal</td>
<td>2</td>
<td>1.030</td>
</tr>
<tr>
<td>Japanese</td>
<td>Premenopausal</td>
<td>3</td>
<td>1.065</td>
</tr>
<tr>
<td>Japanese</td>
<td>Early perimenopausal</td>
<td>1</td>
<td>1.002</td>
</tr>
<tr>
<td>Japanese</td>
<td>Early perimenopausal</td>
<td>2</td>
<td>1.017</td>
</tr>
<tr>
<td>Japanese</td>
<td>Early perimenopausal</td>
<td>3</td>
<td>0.995</td>
</tr>
<tr>
<td>Chinese</td>
<td>Premenopausal</td>
<td>1</td>
<td>1.017</td>
</tr>
<tr>
<td>Chinese</td>
<td>Premenopausal</td>
<td>2</td>
<td>1.043</td>
</tr>
<tr>
<td>Chinese</td>
<td>Premenopausal</td>
<td>3</td>
<td>1.037</td>
</tr>
<tr>
<td>Chinese</td>
<td>Early perimenopausal</td>
<td>1</td>
<td>0.777</td>
</tr>
<tr>
<td>Chinese</td>
<td>Early perimenopausal</td>
<td>2</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Japanese and Chinese models were adjusted for age (years), menopausal status (early perimenopausal vs. premenopausal), dietary calcium intake (mg/day), dietary alcohol intake (kcal/day), dietary protein (kcal/day), height (cm), weight (kg), current smoking (yes/no), physical activity (home, active living, and recreational), and duration of residence in the United States (years). Japanese models were also adjusted for history of hyperthyroidism (yes/no) and included a significant interaction between menopausal status and genistein intake.

† Early perimenopausal defined by self-report of menstruating in the past 3 months but having less-predictable menstrual cycles.

‡ BMD, bone mineral density.

§ BMD values are based on the spine sample; results did not differ for the hip sample.

\( p \) value shown is for the \( F \) test that distributions of BMD values vary by tertile of genistein intake.

\( # \) \( p \) value for interaction between menopause stage and genistein was 0.083 at the spine and 0.006 at the femoral neck. \( p \) values for pairwise comparisons of mean spine BMD values in tertiles of genistein intake among premenopausal Japanese women were as follows: 3 vs. 1 = 0.007; 3 vs. 2 = 0.18; 2 vs. 1 = 0.09. \( p \) values for pairwise comparisons of mean spine BMD values in tertiles of genistein intake among early perimenopausal Japanese women were as follows: 3 vs. 1 = 0.84; 3 vs. 2 = 0.50; 2 vs. 1 = 0.59. \( p \) values for pairwise comparisons of mean femoral neck BMD values in tertiles of genistein among premenopausal Japanese women were as follows: 3 vs. 1 < 0.0001; 3 vs. 2 = 0.17; 2 vs. 1 = 0.001. \( p \) value for pairwise comparisons of mean femoral neck BMD values in tertiles of genistein among early perimenopausal Japanese women were as follows: 3 vs. 1 = 0.65; 3 vs. 2 = 0.33; 2 vs. 1 = 0.53.

was associated with higher adjusted BMD only in Japanese women; no relation was found among the Chinese women \((p = 0.0204\) for genistein-by-ethnicity interaction). At the spine, the genistein-ethnicity interaction was also statistically significant \((p = 0.0284)\).

Although this study focused on a nutrient-level analysis, genistein and daidzein food sources among the Japanese and
Chinese SWAN participants are summarized in appendix table 1. Fermented soy foods (listed in italics) were more highly represented in the Japanese compared with the Chinese women’s diets.

**DISCUSSION**

In this sample of Chinese and Japanese women aged 42–52 years, the relation between dietary genistein intake and bone density was heterogeneous: a positive, dose-response association between genistein and BMD was observed for premenopausal, but not perimenopausal, Japanese women. In contrast, no effect of genistein on BMD was evident in Chinese women. Because the estimated genistein consumption by Japanese women was greater than that of Chinese women, it was important to discern whether the apparent ethnic difference in the effect of genistein on BMD was due to variation in the dose of genistein or to an ethnic difference in physiologic response to genistein. Results of the combined model including both Chinese and Japanese women supported the latter conclusion. We were unable to pursue the possible bone effects of genistein in Caucasian and African-American women because of very low levels of consumption. Important to the interpretation of these results is the colinearity between the two major soy isoflavones, genistein and daidzein, and the fact that soy foods are also rich in other nutrients that we did not measure.

To compare our results with those found in the literature, the capability of the FFQ to estimate genistein intake must be addressed. It is acknowledged that absolute quantities of nutrients consumed cannot be calculated from FFQs. Furthermore, errors in existing databases of the isoflavone content of foods, missing isoflavone food sources, and known small-area variation in the isoflavone content of soy crops combined to limit our confidence in the accuracy of any dietary estimation method of isoflavone intake (11, 12). In our study, the relative ranking of genistein intake should not have been impacted by these constraints; however, because of these known problems with estimation, direct genistein exposure should not be compared with that found in other studies. Genistein dose-response contrasts should be interpreted very cautiously and primarily in relative terms. Finally, because genistein and daidzein are highly correlated, only one of them could be used in mathematical analyses. Because genistein and daidzein are both metabolically active, and most interventional research has administered genistein and daidzein in combination, our data on dietary exposure to isoflavones can be roughly compared with prior work by using our aggregate median values of dietary genistein and daidzein—about 12 mg/day in Japanese women and 5 mg/day in Chinese women.

When what is generally considered the moderate-to-high dose range of genistein and daidzein is used (16), animal and human intervention studies corroborate a positive relation between isoflavones and BMD (23, 24). Fanti et al. tested the ability of three genistein doses (1 µg, 5 µg, and 25 µg per gram of body weight) to prevent ovariectomy-induced bone loss in rats; the lowest dose was ineffective, while the higher two were equally effective (23). Similarly, a 24-week, randomized controlled trial compared the bone density effects of two doses of isoflavones (predominantly genistein and daidzein) in postmenopausal women (24). No BMD effect was found in the women who received 56 mg/day of genistein/daidzein, but those randomized to 90 mg/day of genistein/daidzein evidenced an approximate 2 percent increase in spinal BMD compared with baseline measurements. Consonant results emerge from two other studies that tested single doses of predominantly genistein and daidzein isoflavone compounds (25, 26). In a 2-year study of ovariectomized monkeys, 28 mg of isoflavones daily did not prevent postophorectomy bone loss (25). However, a 24-week intervention with an isoflavone dose of 80.4 mg/day preserved BMD in perimenopausal women (26). In aggregate, although dose-response curves are not readily comparable across species, these intervention studies suggest that above some threshold dose, the isoflavones genistein and daidzein have a positive effect on BMD. Our results in premenopausal Japanese women further point to the possibility of a dose-response relation, with higher amounts of genistein leading to higher BMD.

Results of models that included Chinese and Japanese women and considered an interaction term between ethnicity and genistein supported the hypothesis that these two groups of women have different BMD responses to similar amounts of dietary genistein. How might ethnicity modify the bone tropism exerted by genistein? Japanese women could have a higher density of osteoblast estrogen receptors compared with Chinese women and thus have a greater bone effect for a given dose. To our knowledge, evidence for ethnic differences in estrogen receptor density in bone cells is lacking, but ethnic variations have been reported for the classic estrogen receptor in breast tissue (27, 28). Second, the Japanese diet may contain more readily absorbed forms of isoflavones. It has been proposed that isoflavone aglycones (forms devoid of a sugar moiety) are absorbed faster and in greater amounts than are the glycoside (with sugar attached) forms (29). Compared with the Chinese diet, the traditional Japanese diet is higher in fermented soy products, such as tempeh, miso, and soy paste; fermented soy sources are rich in aglycones (30). Correspondingly, in our sample, fermented soy food sources represented a vastly greater proportion of isoflavone intake for Japanese compared with Chinese women. It is also plausible that, compared with Chinese women, Japanese women may have higher amounts of the β glucosidase enzyme. If Japanese women consume more readily absorbed forms of phytoestrogens or metabolize them more efficiently, then estimates of physiologic exposure based purely on dietary content will underestimate effects of isoflavones in Japanese women.

Bone-trophic effects of phytoestrogens are biologically plausible; mechanisms of action include binding to estrogen receptors, stimulating formation of sex hormone-binding globulin, and inhibiting tyrosine kinase (31, 32). However, our analysis explored more than the simple hypothesis that genistein and daidzein would benefit bone; we postulated the more complex theory that the bone effect of phytoestrogens might vary according to a woman’s menopause status.
a proxy for her endogenous hormonal milieu. This idea for effect modification by endogenous hormone status derives from the concept that phytoestrogens are selective estrogen receptor modulators. They demonstrate differential binding and activity to the alpha and beta estrogen receptors (32) based on interactions with the receptor binding pocket and the resultant conformational change of the receptor (33). Given that phytoestrogen-estrogen receptor interactions will necessarily compete with those of the cognate ligand, the result will likely depend in part on concentrations of endogenous sex steroids (9). The present study used self-reported premenopausal (defined as regular menses) status and early perimenopausal (defined as less-predictable menses) status as proxies of endogenous sex steroids and found a positive effect of genistein on BMD only in regularly menstruating women. Additional work in SWAN, using actual measured hormone levels, may be able to explore this observation further.

Given the lack of a criterion standard to measure diet, the validity of nutrient estimates based on an FFQ should be considered in the context of this analysis. Measurement of genistein exposure in SWAN relied on a modification of the Health Habits and History Questionnaire (“Block FFQ”) (18). The FFQ was adapted to accommodate Asian-style mixed dishes and was programmed to calculate genistein and daidzein intakes based on a comprehensive compilation of published food sources (12). Concordant with other diet measurement techniques (34), this study found that consumption of genistein and daidzein was almost perfectly correlated. We assessed the validity of the daidzein and genistein estimates computed from the modified Block FFQ in an independent sample of 58 Japanese and 18 Caucasian volunteers (35). Four 24-hour urine collections were made, and high-pressure liquid chromatography (HPLC) determinations of daidzein and genistein were made in each. The Spearman correlation between the four urinary assays of daidzein and the FFQ-estimated daidzein intake was 0.49, and the urinary-FFQ correlation for genistein was 0.30. These correlations are higher than many others that have been reported between dietary measures and biomarkers (36–38) and are certainly in the range considered adequate for ranking of nutritional intakes (39).

The foremost limitation of this study is the incompleteness of the database of isoflavone food sources (11, 12), which may have led to underestimation of true intake; however, this shortcoming should not have diminished our ability to rank participants. We may have captured the phytoestrogen food sources of Japanese women more completely than those of Chinese women, producing biased estimates. Nonetheless, negatively biased estimates for the Chinese women would not explain the lack of genistein’s effect on BMD in Chinese women in the combined Japanese and Chinese model. It must be acknowledged that the modified Block FFQ (18) was not validated against urine or serum estimates of phytoestrogens in Chinese women. It is also possible that unmeasured dietary constituents or confounders could have been responsible for our results. This analysis was cross-sectional, which constrained causal inference. Finally, because our finding of a positive relation between dietary genistein and BMD was confined to only the premenopausal subgroup of Japanese women, these results may be chance findings produced by multiple statistical testing.

In summary, this study found a strong, positive, dose-response relation between dietary soy isoflavones (assessed by genistein) and BMD in premenopausal Japanese women. It also suggests that the absent effect of soy isoflavones on BMD in Chinese women was not due to their lower average intake compared with Japanese women and that dietary differences in fermented soy food sources may be one reason for this ethnic difference. Further work is needed to confirm these provocative findings and to understand the mechanisms underlying these complex relations.

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REFERENCES


Appendix table 1 follows.
APPENDIX TABLE 1. Food sources of genistein and daidzein among Chinese and Japanese participants in the Study of Women's Health Across the Nation,* United States, 1996–1997

| Item                        | Chinese Average daily nutrient (µg)† | % of total‡ | Japanese Average daily nutrient (µg) | % of total
|-----------------------------|-------------------------------------|-------------|-------------------------------------|-------------
| Genistein                   |                                     |             |                                     |             |
| Tofu, bean curd             | 4,512                               | 70%         | Tofu, bean curd                      | 4,384       | 33%         |
| Soy milk                    | 961                                 | 15%         | Soybeans,                            | 2,689       | 21%         |
| Soybean sprouts             | 555                                 | 9%          | Soybeans, fermented§                 | 1,832       | 14%         |
| Meat substitute             | 178                                 | 3%          | Soybeans, fresh green                | 2,375       | 18%         |
| Soybean paste               | 101                                 | 2%          | Miso soup§                           | 943         | 7%          |
| Miso soup§                  | 86                                  | 1%          | Soybeans, roasted                    | 723         | 5%          |
| Soy sauce                   | 22                                  | <1%         | Aburage/atsuage                       | 139         | 1%          |
| Fermented bean curd§        | 16                                  | <1%         | Soy milk                             | 94          | <1%         |
|                            |                                     |             | Meat substitute                       | 64          | <1%         |
|                            |                                     |             | Tofu, dry spiced                     | 62          | <1%         |
|                            |                                     |             | Soy sauce                            | 30          | <1%         |
|                            |                                     |             | Fermented bean curd§                 | 2           | <1%         |
|                            |                                     |             |                                     |             |
| Daidzein                    |                                     |             |                                     |             |
| Tofu, bean curd             | 2,066                               | 62%         | Tofu, bean curd                      | 2,621       | 28%         |
| Soy milk                    | 666                                 | 20%         | Soybeans, fermented§                 | 2,127       | 23%         |
| Soybean sprouts             | 333                                 | 10%         | Soybeans, fresh green                | 2,007       | 21%         |
| Meat substitute             | 94                                  | 3%          | Miso soup§                           | 1,296       | 14%         |
| Soybean paste               | 63                                  | 2%          | Soybeans, roasted                    | 674         | 7%          |
| Miso soup§                  | 61                                  | 2%          | Aburage/atsuage                       | 435         | 5%          |
| Soy sauce                   | 36                                  | 1%          | Soy milk                             | 96          | 1%          |
| Fermented bean curd§        | 14                                  | <1%         | Koritofu                             | 60          | <1%         |
|                            |                                     |             | Meat substitute                       | 48          | <1%         |
|                            |                                     |             | Tofu, dry spiced                     | 37          | <1%         |
|                            |                                     |             | Soy sauce                            | 23          | <1%         |
|                            |                                     |             | Fermented bean curd§                 | 2           | <1%         |

* Values are for all Japanese (n = 274) and Chinese (n = 250) women who completed a food frequency questionnaire.
† Values for genistein and daidzein were estimated by using the modified Block food frequency questionnaire (FFQ) (Am J Epidemiol 1986;124:453–69). The genistein and daidzein database used in that FFQ was derived from the compendium published by Rienli and Block (Nutr Cancer 1996;26:123–48).
‡ Values for nutrient intake and percentages were rounded to the nearest integer; percentages do not sum to 100 because of rounding.
§ Food contains fermented soy products.