Dietary Soy Intake and Urinary Isoflavone Excretion among Women from a Multiethnic Population

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Abstract
Isoflavones are present in soybeans and its products in concentrations up to 300 mg/100 g, have estrogenic and antiestrogenic properties, and may be protective against hormone-related cancers. The purpose of this cross-sectional study was to investigate the association between urinary isoflavone excretion and self-reported soy intake. A total of 102 women of Caucasian, Native Hawaiian, Chinese, Japanese, and Filipino ancestry completed a dietary questionnaire for soy products consumed during the last year and during the 24-h period before urine collection. Overnight urine samples were analyzed for coumestrol and the soy isoflavones genistein, daidzein, and glycitein and their main human metabolites by reverse-phase high-pressure liquid chromatography. Soy protein and isoflavone intake (predominantly from tofu) were estimated using published nutritional databases. Wilcoxon's rank-sum test scores and Spearman rank correlation coefficients were computed. Japanese women excreted more daidzein, genistein, and glycitein than did Caucasian women, whereas Caucasian women excreted slightly more coumestrol. Soy intake differed significantly among ethnic groups. Dietary soy protein and isoflavone intakes during the previous 24 h were positively related to urinary isoflavone excretion \( r_s = 0.61 \) (\( P < 0.0001 \)) and 0.62 (\( P < 0.0001 \)), respectively. Urinary excretion of isoflavones was also related to annual dietary soy protein and isoflavone intake \( r_s = 0.32 \) (\( P < 0.0012 \)) and 0.31 (\( P < 0.0016 \)), respectively. The strong correlation between urinary isoflavone excretion and self-reported soy intake validates the dietary history questionnaire that is now used in a study exploring dietary risk factors for breast cancer.

Introduction
The low risk for breast and prostate cancer in Asian populations combined with evidence from migration studies suggests that environmental factors such as diet may influence the occurrence of these diseases (1–5). From 1988 to 1992, breast cancer incidence for ethnic groups in the United States (per 100,000 women, age-adjusted to the 1970 United States population) was as follows: Caucasians, 111.8; Native Hawaiians, 105.6; Japanese, 82.3; Chinese, 55; and Filipino, 73.1 (6). These variations have been attributed to differences in dietary fat and fiber intake and possibly to soy consumption among Asian women (7–9). The interest in dietary soy has arisen from findings related to the potential cancer-protective properties of isoflavones (10, 11). Isoflavones are plant products with estrogenic activity and are therefore phytoestrogens, which include a wide variety of phytochemicals such as coumestans and lignans (12, 13). The term phytoestrogen was coined after the observation that Australian sheep grazing on a certain type of subterranean clover had a high rate of infertility (14), an observation later attributed to the high isoflavone content of this plant. The isoflavones have a heterocyclic phenol structure that closely resembles estrogen and enables them to bind to estrogen receptors and to exert weak estrogenic effects (13, 15–17). They may act as antiestrogens by competing with endogenous estrogen for receptor binding, thereby possibly reducing the risk for breast cancer by decreasing the promotional effects of high levels of endogenous estrogens (10, 18, 19) or by altering estrogen biosynthesis (20, 21). Alternative mechanisms suggested for isoflavones to prevent cancer and other chronic conditions are modification of enzyme activity, such as topoisomerase or tyrosine protein kinases (22) that play a role in cell proliferation and transformation, as well as breast cancer oncogene expression (23), inhibition of angiogenesis (24), and apoptosis (25).

Soybeans contain high amounts (concentrations of up to 100–300 mg/100 g) of the glycosides of the isoflavones daidzein, genistein, and glycitein (26–29). Daidzein present in soy foods is partially converted by the gut flora into equal and DMA as end products (30, 31). The other common dietary isoflavone, genistein, is converted to p-ethylphenol (31). The uncertain metabolism of soy isoflavones led to the identification of several minor intermediate metabolites (32). The isoflavones and their metabolites can be measured in food, plasma, urine, and feces using gas chromatography and HPLC (27, 33–41).

Studies among various populations and dietary groups have shown large amounts of isoflavonoids in the plasma, urine, and feces of vegetarians; macrobiotics; and in Japanese individuals consuming a traditional Japanese diet (37, 38). Whereas substantial variations among individuals in the excretion of isoflavones and their metabolites have been described (39), information on ethnic differences in soy intake and the excretion of urinary isoflavones is limited (42). Hawaii is an excellent location to explore the possible effects of soy product consumption, due to its multiethnic population with a high

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1 The abbreviations used are: DMA, O-desmethylyangolensin; HPLC, high-pressure liquid chromatography; i.d., inside diameter.
proportion of women of Asian ancestry who have maintained some of their traditional dietary habits. The objectives of this cross-sectional study were to explore the variation in dietary soy intake and urinary isoflavone excretion among women of Japanese, Filipino, Native Hawaiian, Chinese, and Caucasian ancestry and to investigate the association between dietary soy intake and urinary isoflavone excretion.

Materials and Methods

Study Population. Hawaii has a multiethnic population with approximately 24% Caucasian, 20% Japanese, 11% Filipino, 19% Native Hawaiian, 5% Chinese, and 21% other ethnic groups (43). Participating women were recruited from two large mammography clinics in Honolulu. The institutional review boards at all participating organizations approved the study protocols. Women interested in participating received dietary questionnaires and consent forms with addressed return envelopes by mail. In appreciation for their cooperation, women were sent a summary of their dietary intake of various macro- and micronutrients at the end of the study. Mammograms for all participating women were obtained from the clinics after the radiological evaluation was completed. Women with a history of breast cancer, a previous breast biopsy or surgical procedure, or a mammogram with a suspicious lesion requiring biopsy were excluded from the study. Antibiotic use or other metabolic conditions that might affect isoflavone uptake or metabolism were not assessed.

Study participants with mixed ancestry were classified using both parents’ ancestry and the following rules. If a person’s ethnicity is recorded as a combination of Native Hawaiian and any other ethnicity, the summary code is Native Hawaiian. Otherwise, the summary code is the first stated non-Caucasian ethnicity. Although all Native Hawaiian women were of mixed ancestry, the majority (84%) of Asian women reported one ancestry only.

Dietary Assessment. A self-administered questionnaire was used to obtain demographic and reproductive information and a family history for each participant. The dietary history questionnaire collected the usual frequency of consumption and portion size for more than 50 food items, alcoholic and nonalcoholic beverages, and vitamin supplements during the previous year. A separate questionnaire listing 12 soy-based foods with local names was used to collect information on soy consumption. We used United States Department of Agriculture Food Composition tables (44) to compute soy protein intake and used isoflavone data from food analyses to estimate isoflavone intake (45).

Urine Collection. An overnight urine sample was collected for 102 study participants, and a 24-h dietary questionnaire for soy-based foods was obtained at the same time. Of 129 women eligible for urine collection, 5 women refused to provide a sample, 2 women were menstruating, 8 women were on vacation, 3 women lived too far away, and 9 women could not be reached after repeated calling, resulting in a participation rate of 78%. A container with 0.2 g of ascorbic acid and 0.3 g of boric acid (to prevent bacterial contamination and degradation of analytes) was delivered to each woman. Women were instructed to collect the first urine sample in the morning and all of the samples if they urinated during the night after going to bed. They were asked to record the times of last urination before going to bed and last urine collection in the morning. The samples were stored in the participants’ refrigerators until pick-up in the morning and transported to the laboratory on ice. After mixing and weighing, each urine sample was transferred to three 25-ml disposable plastic tubes and stored at ~70°C until analyzed.

Urine Analysis. Urine samples were analyzed by diode array reverse-phase HPLC for soy isoflavones, their most common mammalian metabolites, and coumestrol (29). In brief, frozen urine samples were thawed, vortex-mixed, and centrifuged at 850 × g for 5 min. A 2.0-ml clear supernatant was mixed with 0.4 ml of 0.5 M triethyl-amine acetate buffer (pH 7.0) and 20 μl of flavone (120 ppm in 96% ethanol) as an internal standard. The mixture was incubated with 10 μl of β-glucuronidase (Boehringer # 127680; 200 units/ml, 0.1 μmol/5 μl) and 10 μl of arylsulfatase (Boehringer # 102890; 5 units/ml) for 1 h at 37°C in the dark followed by repeated extraction with diethyl ether. The combined organic phases were dried under nitrogen and redissolved in 100 μl of methanol by sonication for 10–20 s and in 100 μl of 0.2 M sodium acetate buffer (pH 4.0). After centrifugation, 20 μl of the clear solution were injected into the HPLC system. The internal standard isoflavone was analyzed in the same way in the same batch as urine extracts for internal standard recovery calculation purposes. HPLC analyses were carried out on a NovaPak C18 (150 × 3.9 mm, inside diameter; 4 μm) reverse-phase column (Waters, Milford, MA) coupled to an Adsorbosphere C18 (10 × 4.6 mm, inside diameter; 5 μm) direct connect guard column (Alltech, Deerfield, IL). Elution was performed at a flow rate of 0.8 ml/min with the following step gradient: (a) 20% acetonitrile in acetic acid–water (10:90, v/v) for 16 min; (b) 70% acetonitrile for 14 min; and (c) 20% acetonitrile for 10 min. Analytes were monitored with a dual-channel diode array detector at 260 nm during the entire HPLC run, at 280 nm during equal elution, and at 342 nm during coumestrol elution. Observed peaks were scanned between 190 and 400 nm. These phytoestrogens were identified by comparing retention times and UV absorption patterns with authentic standards analyzed in the same batch and with reported UV data (46). Concentrations of analytes in the urine were calculated with area units obtained from HPLC analyses and the slope of the calibration curve. The excretion rates are expressed in nanomoles/hour after consideration of urine volume and adjustment for the time between last urine collection and previous void and internal standard recovery. Detection limits were 0.2 nmol/l for genistein, 0.4 nmol/l for daidzein and glycitein, 0.9 nmol/l for equol, 1.4 nmol/l for DMA, and 0.5 nmol/l for coumestrol. On 10 randomly selected urine samples, a repeat analysis was performed to test reliability of measurements.

Statistical Analysis. After examining each variable separately for the normality of the distribution, we computed Spearman rank correlation coefficients (47) to evaluate the relationship between urinary isoflavone excretion and dietary intake of isoflavones and soy protein consumption during the previous 24 h and during the previous year. Because of the non-normality of these distributions, we calculated k-statistics (47) to verify the correlations. A mean coefficient of variation was computed for the 10 urine samples with repeat measurements by dividing the ratio of the SD and the mean of each repeat sample by 10 (47). Nonparametric analyses (47) were performed to evaluate differences among all ethnic groups (Kruskal-Wallis test) and between two ethnic groups (Wilcoxon scores). Statistical analysis was performed using the SAS statistical software package version 6.12 (SAS Institute, Inc., Cary, NC).

Results

The study population included 42 Caucasian women, 25 Japanese women, 13 Chinese women, 11 Native Hawaiian women,
The mean urinary excretion for isoflavones was more than 7.1 times higher in Japanese women than in Caucasian and Filipino women. Likewise, women with Japanese, Chinese, or Native Hawaiian ancestry excreted more isoflavones in urine than did Caucasian and Filipino women. All of the ethnic groups investigated excreted daidzein at the highest rate, followed by genistein and glycitein (Table 1; Fig. 2). Equol was excreted at the highest rate by Chinese women, whereas all other women excreted coumestrol at a very low rate. Coumestrol was excreted at higher rates by Caucasian women than it was by any other group.

The mean dietary intake of soy protein during the previous year was three times higher in Japanese women than it was for Caucasian women (Table 1). Soy consumption for Chinese and Native Hawaiian women was more than twice as high as that in Caucasian women. Filipino women and the women of other ethnicities had the lowest mean dietary intake of soy protein. Whereas only 1 Japanese woman did not excrete any isoflavones, 16 (38%) Caucasian women, 3 (27%) Native Hawaiian women, 1 (25%) woman of other ethnicity, 2 (15%) Chinese women, and 1 (14%) Filipino woman did not excrete any isoflavones. For all ethnic groups, daidzein was excreted at the highest rate, followed by genistein and glycitein (Table 1; Fig. 2). The excretion of urinary isoflavonoid metabolites and coumestrol showed great variation among ethnic groups (Table 1; Fig. 2). Equol was excreted at the highest rate by Chinese women, whereas all other women excreted coumestrol at a very low rate. Coumestrol was excreted at higher rates by Caucasian women than it was by any other group.

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Discussion

In this cross-sectional study, we examined urinary isoflavonoid excretion and soy intake in 102 healthy women from different ethnic groups. Japanese, Chinese, and Native Hawaiian women were found to consume higher amounts of soy protein than were Caucasian and Filipino women. Likewise, women with Japanese, Chinese, or Native Hawaiian ancestry excreted more isoflavones in urine than did Caucasian and Filipino women. All of the ethnic groups investigated excreted daidzein at the highest rate, followed by genistein. This is in good agreement with previous reports on urinary isoflavonoid excretion (39, 40, 48-50). Glycitein was excreted at very low levels, and great variation was observed in the excretion of isoflavonoid metabolites in all groups. Urinary isoflavone excretion was significantly related to soy protein intake during the previous 24 h and during the previous year as measured by a food frequency questionnaire.
Fig. 1. Mean soy protein intake and urinary isoflavonoid excretion among 102 multiethnic women. ◼, soy protein intake during the previous 24 h (g/day); ◆, soy protein intake during the previous year (g/day); ●, urinary isoflavones (nanomoles/hour).

Thus far, only a study from California (42) has reported urinary isoflavone excretion by ethnic group. In that study with 50 subjects, no statistically significant difference among various ethnic groups for isoflavone excretion was found, but the small group of Japanese women (n = 5) excreted slightly more isoflavones than did other ethnic groups. Similar to our results, Japanese women excreted very low levels of coumestrol compared with Caucasian women. The comparison between the Californian study (42) and our study is limited by the differences in the ethnic groups (Caucasian, Latina, African American, and Japanese versus Caucasian, Chinese, Filipino, Japanese, and Native Hawaiian) and the fact that 24-h urine samples were collected in California (42). The advantage of our investigation was a larger sample size (102 women) from a multiethnic population in which women of Asian ancestry have maintained some of their traditional dietary habits.

Other studies with small convenience samples have examined urinary phytoestrogen excretion for various populations, related them to different diets (37, 38), and described a great variation in urinary isoflavones and their metabolites. Urinary isoflavone excretion increased as much as 1000-fold (39) when soy was added to a typical Western diet. Similarly, urinary excretion of isoflavonoids was directly related to dose when 11 men and 9 women consumed soy protein in a controlled experimental diet trial (51) or when randomly added to the usual diet (45). Soy doses (grams of beans) correlated excellently with the observed isoflavonoid amounts excreted in urine during the first 24 h when healthy subjects were challenged with different amounts of roasted soybeans (39).

Inter- and intraindividual differences were reported in a study of six women and five men who consumed 44–96 g of roasted soybeans (39). All individuals excreted genistein and daidzein, but some did not excrete any or excreted only low levels of other isoflavonoid metabolites. A similar variation in the excretion of metabolites and coumestrol among various ethnic groups was obtained in our study (Fig. 1). This variability may be due to the nature of gastrointestinal absorption of soy isoflavones and metabolism by intestinal bacteria (34, 52) such as Lactobacilli, Bacteroides, Bifidobacterium, and Clostridia (53). Variations in dietary habits can lead to differences in the gut flora (54). The intestinal bacteria vary widely between individuals, and only selected strains are capable of hydrolyzing plant β-glucosides, such as isoflavones occurring in unfermented soy foods, and performing further flavonoid metabolism by ring fission (55, 56). Evidence for that hypothesis was provided in a well-controlled liquid diet study (52) in which seven women consumed three soy milk meals/day. Recovery of daidzein was significantly greater than that of genistein (P < 0.01) in women excreting small amounts of fecal isoflavones. Anaerobic incubation of isoflavones with human feces showed that the intestinal half-life of daidzein and genistein is 7.5 and 3.3 h, respectively. Lower absorption levels of isoflavones may explain our finding that Japanese, Chinese, and Native Hawaiian women had relatively high intakes of soy protein, but when compared to Japanese women, excretion of urinary isoflavones was low for both Chinese and Native Hawaiian women (Fig. 1). Variations in the time when soy foods were consumed could also be responsible for this observation.

For this study, only one overnight urine sample was collected for easier implementation and higher compliance, resulting in a participation rate of 78%. Franke and Custer (39) recommended a combination of three urine samples collected every other night for each subject. This would allow integration of an individual’s phytosterogen exposure over approximately 1 week, a period that generally reflects usual dietary habits. Multiple 24-h urine samples would be a more valid measurement of isoflavonoid excreted in urine than one overnight urine sample but would make participation more difficult and costly. Another limitation of our study was the poor representation of some ethnic groups. The representation of Native Ha...
Table 2  Correlations between urinary isoflavone excretion and dietary intake of soy protein and isoflavones

<table>
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<th>First variable</th>
<th>Second variable</th>
<th>$r$</th>
<th>$P$</th>
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<td>Urinary isoflavone excretion (nmol/h)</td>
<td>Soy protein intake during previous 24 h (g/day)</td>
<td>0.61</td>
<td>0.0001</td>
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<td>Urinary isoflavone excretion (nmol/h)</td>
<td>Isoflavone intake during previous year (mg/day)</td>
<td>0.31</td>
<td>0.0016</td>
</tr>
<tr>
<td>Urinary isoflavone excretion (nmol/h)</td>
<td>Isoflavone intake during previous 24 h (mg/day)</td>
<td>0.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>Soy protein intake during previous year (g/day)</td>
<td>Soy protein intake during previous 24 h (g/day)</td>
<td>0.53</td>
<td>0.0001</td>
</tr>
<tr>
<td>Isoflavone intake during previous year (mg/day)</td>
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<td>0.0001</td>
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<tr>
<td>Soy protein intake during previous 24 h (g/day)</td>
<td>Isoflavone intake during previous 24 h (mg/day)</td>
<td>0.96</td>
<td>0.0001</td>
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</table>

* Spearman rank correlation coefficient.
Urinary Isoflavone Excretion among Multiethnic Women

This study is one of the first to investigate soy intake and isoflavone excretion with a larger sample size of healthy women of various ethnic origins. Dietary soy protein and isoflavone intake based on self-reporting for the previous 24 h and for the previous year were positively related to the urinary excretion of isoflavones. The results of this study suggest differences in urinary excretion of isoflavones by ethnic group that are related to self-reported dietary soy intake and to differential intestinal absorption patterns. The strong correlation between urinary isoflavone excretion and self-reported soy intake validates the questionnaire that is now used in a study exploring dietary risk factors for breast cancer.

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References

Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population.


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