Neither Background Diet Nor Type of Soy Food Affects Short-Term Isoflavone Bioavailability in Women \(^1,2\)

(ManUScript received 23 July 1999. Initial review completed 20 August 1999. Revision accepted 6 December 1999.)

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**ABSTRACT** To characterize bioavailability of soybean isoflavones, proposed anticarcinogenic food components, eight women, ages 20–41 y, were fed 0.9 mg isoflavones/kg body wt from soymilk at 0730, 1230 and 1730 h for 1 d. Subjects consumed three background diets in random order: a diet prepared for them (basic foods diet) or a self-selected diet at the specified times, or a self-selected diet eaten ad libitum. In a second study, women were fed single isoflavone doses of 0.8–1.4 mg/kg in breakfast casseroles containing tofu, tempeh, cooked soybeans or texturized vegetable protein. Both studies were conducted in randomized, cross-over designs. Plasma, urine and fecal isoflavones were measured by reverse-phase HPLC. After consumption of background diets, 48-h urinary recovery of daidzein (D) was 26–27%, and of genistein (G), 18–20% of the dose given with each diet. At 24 h after consumption of different background diets, plasma D and G concentrations were similar (1.4 ± 0.7 mmol/L) and were not affected by diet selection. Urinary recoveries of D over 24 h from the various soy foods were 38–51%, and of G, 9–16% of the dose given. In both studies, urinary recovery of D was significantly greater than that of G. Only a few percentage of the total isoflavone dose was recovered in feces, probably due to bacterial breakdown of these compounds. Therefore, isoflavone bioavailability may not be affected by choice of background diet or diet source of isoflavones. *J. Nutr.* 130: 798–801, 2000.

**KEY WORDS:** isoflavones, bioavailability, diet selection, soybean foods, humans

Isoflavones are abundant in soybean foods (0.2–1.5 mg/g) (Wang and Murphy 1994). The three isoflavones, daidzein (D)\(^4\), genistein (G) and glycitein, are found in four chemical forms (aglycone, glucoside, acetylglucoside and malonylglucoside) (Kudou et al. 1991). Glycitein is weakly estrogenic (Song et al. 1999), as are G and D (Farmakalidis and Murphy 1985). Estrogen-like activity is one of many proposed anticarcinogenic mechanisms of these compounds (Messina and Barnes 1991). Dietary intake of soybean isoflavones was associated with lower breast and prostatic cancer incidence in Eastern Asia (Adlercreutz et al. 1995). An isoflavone extract providing 240 mg total isoflavones/kg diet significantly suppressed rat hepatocarcinogenesis initiated by diethylnitrosamine and promoted by phenobarbital (Lee et al. 1995). Rats consuming a soy-based diet developed fewer mammary tumors following administration of the carcinogens N-methylnitrosourea and 7,12-dimethylbenz[a]-anthracene than rats fed isonitrogenous and isocaloric diets without soy (Barnes et al. 1990). G inhibited the growth of estrogen receptor-negative or -positive human breast cancer cell lines [50% inhibitory concentration (IC\(_{50}\)) = 24–44 mmol/L; Peterson and Barnes 1991] and inhibited activation of tyrosine kinases (IC\(_{50}\) = 3–100 mmol/L; Akiyama et al. 1987). D and G (IC\(_{50}\) of 8 and 2.2 mmol/L, respectively) inhibited production of inositol phosphates, key intracellular signals of proliferation stimulated by AlF\(_4\) in 3T3 cells (Higashi and Ogawara 1992). These findings suggest that isoflavones in soybean foods might play a role in human cancer protection.

Characterization of isoflavone bioavailability in humans is needed to assess the role of these compounds in cancer risk reduction. Previous human feeding studies showed that absorption of isoflavones from soymilk in a controlled liquid diet was dose-dependent (Xu et al. 1994, 1995). But, people eat a variety of soybean foods and background diets. Fermentation increases the isoflavone aglycone content of soy foods, and processing alters the type of isoflavone glycoside. Therefore, soy food type might alter human isoflavone bioavailability.

These studies address this hypothesis. Furthermore, it is of practical concern in studying the biological effects of these compounds to determine if carefully defined diets limit inter- and individual variability in isoflavone bioavailability compared with diets consumed ad libitum.

**MATERIALS AND METHODS**

**Subjects and protocol.** The procedures for this feeding study were approved by the Human Subjects Committee of Iowa State University (ISU). Written informed consent of subjects was obtained. All subjects were omnivorous and in good health, based on medical histories and physical examinations performed by Student Health Center physicians at ISU. Subjects did not take any medications including antibiotics during the feeding studies.

**Expt. 1.** Eight women between 20 and 41 y of age, with body weight of 58.4 ± 10.5 kg and body mass index of 21.4 ± 2.2 kg/m\(^2\) (means ± SD), were fed three servings of isoflavones per day (0.9

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\(^2\) Journal paper No. J-17673 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA Project No. 3075, supported by Hatch Act and State of Iowa funds, National Institutes of Health grant CA 56308–02, and the Center for Designing Foods to Improve Nutrition, Iowa State University.

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\(^4\) Abbreviations used: D, daidzein; G, genistein; IC\(_{50}\), concentration causing 50% inhibition of action; ISU, Iowa State University; TVP, texturized vegetable protein.
mg/kg body weight in each meal) from soymilk powder (Now Foods, Glendale Heights, IL) reconstituted with distilled water. The three doses of soy milk were served at 0730, 1230 and 1730 h, with each day of dosing separated by a 1-wk washout period. Subjects were asked not to consume anything for 10 h before soy milk dosing. Different background diets were consumed in a randomized cross-over design. A basic-foods diet was consumed at 0730, 1230 and 1730 h, with the soy milk dosed. This diet consisted of a toasted plain bagel, 14 g grape jelly, and 250 mL orange juice at 0730 h; two slices white bread, 28 g Swiss cheese (KraftTM, Glenview, IL), mustard, one medium apple, 15 regular potato chips (28 g, Pringle'sTM, Procter & Gamble, Cincinnati, OH) and 250 mL water at 1230 h; two slices white bread, 14 g Colby Jack cheese (KraftTM), mustard, 15 tortilla chips (12 g; Tostitos™ Frito-Lay, Plano, TX), 254 mL orange juice at 1730 h, 22 honey-flavored Teddy GrahamSTM (28 g; Nabisco, Parsippany, NJ) and a medium apple at 1930 h. Subjects eating the basic foods diet were instructed not to eat anything other than what was given on the feeding day. When the subjects ate a self-selected diet, they chose their own foods but consumed them at the same time as the soy milk dosed. When the subjects ate ad libitum, they chose their own foods and meal times, but soy milk was consumed at the same times as with the other feeding regimens. Subjects consuming self-selected and ad libitum diets were instructed to completely record their food and beverage intakes during each feeding day. Dietary data were analyzed by Nutritionist IV version 2.0 (N-Squared Computing, Salem, OR).

Expt. 2. The subjects were 10 women between 20 and 35 y of age, with body weight of 59.6 ± 6.0 kg and body mass index of 21.6 ± 1.2 kg/m². Subjects were fed four types of soybean foods: tofu, texturized vegetable protein (TVP), tempeh or cooked soybeans. Subjects were instructed not to eat anything other than what was given on the feeding day. Soy foods were given at breakfast in a baked caserole consisting of the soy food (~30 g TPV, 100 g tempeh or cooked soy beans or 300 g tofu, providing approximately equal amounts of isoflavones from each food) mixed with one large egg, 40 g sharp cheddar cheese (KraftTM) and 70 g salsa (PaceTM Thick & Chunky, San Antonio, TX), one slice white bread, one cup orange juice, and 250 mL water at 0730 h and other meals exactly as for the basic-foods diet in expt. 1. The controlled diet was designed to meet subjects' average energy requirement according to the Recommended Dietary Allowances tables (Food and Nutrition Board 1989). Feeding days were separated by 1-wk washout periods. Isoflavone dose ranged from 0.8–1.4 mg/kg body weight based on HPLC analysis of the soy foods ingested. Data from soy foods fed separately (six at the first feeding and five at the second feeding) were not used because packages of those commercial soy foods were mixed in.

In both studies, during the washout periods subjects were asked not to eat any foods from a list of items which may contain isoflavones. No adverse effects such as diarrhea were reported after soy food dosing.

Results. Isoflavone concentrations in soymilk powder was 1.25 ± 0.08 mg/g. G and D were 56.0 and 44.0%, respectively, of the molar total of the two isoflavones. Soy food isoflavone concentrations were: tofu, 0.46 ± 0.09 mg/g (57:43, G/D); tempeh, 0.64 ± 0.02 mg/g (60:40, G/D); TVP, 0.42 ± 0.01 mg/g (54:46, G/D); and cooked soybean, 0.58 ± 0.02 mg/g (54:46, G/D). D, G and equal recoveries from spiked samples of urine, plasma and feces were 80–92, 64–78 and 57–72%, respectively. The amount of urinary isoflavones for all background diets was about four times greater in the first 24 h after dosing than in the second 24 h after dosing, 20 ± 8 mg D and 9 ± 7 mg MA). Each dry fecal sample (10 g) was stored in a -20°C freezer until analysis.

Soy milk powder and soy foods analysis. The concentrations of total isoflavones in soy milk powder and four soy foods were measured as isoflavone aglycones after hydrolysis in 1 mol/L HCl at 98°C (Wang et al. 1990). HPLC analysis was as described by Wang and Murphy (1994). Samples were analyzed in triplicate.

Biological sample analysis. Sample preparation for plasma and urine isoflavone analysis was performed according to the methods described by Lundh et al. (1988). Plasma and urine samples were treated with glucuronidase/sulfatase (Sigma Chemicals Company, St. Louis, MO) to produce isoflavone aglycones. Fecal samples were prepared as described by Xu et al. (1994). Chromatographic analysis of plasma, urine and fecal samples was as described by Xu et al. (1994). Samples were injected with a Spectra-Physics Autosampler Model 8780XR (Spectra-Physics, Fremont, CA). Isoflavones were separated over a 3.9 nm i.d. x 30 cm length µ-Bondapak C18 reverse-phase column (Waters-Millipore, Bedford, MA) with gradient elution at ambient temperature. A Beckman (Fullerton, CA) HPLC system, including two Model 110B pumps, one Model 420 Microprocessor solvent flow controller, with a Model 163 variable wavelength detector set at 254 nm connected with a Model 427 integrator was used.

Daidzein (4',7-dihydroxyisoflavone) was obtained from Life Science Group, ICN Pharmaceuticals, Plainview, NY; G (4',5,7-trihydroxyisoflavone) from Calbiochem Corporation, La Jolla, CA and equol (4',7-isoflavandiol) was a generous gift from Dr. H. Adlercreutz (Department of Clinical Chemistry, University of Helsinki, Finland). Recovery was measured by spiking plasma, urine and fecal samples randomly with D, G or equol (100 µL, 1.5 mg/L).

Statistical methods. ANOVA (General Linear Model) was performed with the Statistical Analysis System (SAS Institute, Cary, NC) version 6.06 on the Iowa State University mainframe computer to evaluate nutrient composition of subjects' diets and isoflavone bioavailability. Values are means ± SD. A P-value of 0.05 or less was considered to be significant. Subjects and feeding times were treated as blocks. Percentage recovery of isoflavone from urine and feces and plasma isoflavone concentrations over time were compared among treatments and between G and D. If the effect from any factor other than type of isoflavone was significant, Tukey's test was used for comparison of means within the factor.
was 1% of the ingested amount.

Table 3 for D and about 14% for G (for foods tested (dosing did not differ with soy food type (cooked soybean, TVP, 
creted. Urinary recoveries of D and G over 24 h after soy 
groups fed the other foods. During feeding of the four soybean 
% of the ingested total isoflavone dose was ex-

Plasma isoflavone concentration of D or G was significantly 
percentage of ingested dose was significantly greater than genistein 

Expt. 2. Protein intake when subjects consumed TVP was 
soy foods, 21–34% of the ingested total isoflavone dose was 
Urinary recoveries of D and G over 24 h after soy 
Do not differ at either time after dosing and did not 

Expt. 2.

G excreted from 0 to 24 h vs. 3 ± 3 mg D and 4 ± 5 mg G 
Plasma isoflavone concentration of D or G was significantly 

G excreted from 24 to 48 h (P < 0.05). After 48 h, urinary 


TABLE 1

<table>
<thead>
<tr>
<th>Protein, g</th>
<th>Basic food</th>
<th>Self-selected</th>
<th>Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 ± 3a</td>
<td>81 ± 7b</td>
<td>85 ± 6b</td>
<td></td>
</tr>
<tr>
<td>Total fat, g</td>
<td>44 ± 2a</td>
<td>57 ± 5b</td>
<td>63 ± 6b</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>54 ± 0a</td>
<td>127 ± 7b</td>
<td>118 ± 6b</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>15 ± 0a</td>
<td>24 ± 5b</td>
<td>25 ± 2b</td>
</tr>
<tr>
<td>Monounsaturated fat, g</td>
<td>23 ± 1a</td>
<td>27 ± 3b</td>
<td>31 ± 3c</td>
</tr>
<tr>
<td>Soluble dietary fiber, g</td>
<td>2.5 ± 0.1</td>
<td>2.7 ± 0.7</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>Insoluble dietary fiber, g</td>
<td>9.2 ± 0.3</td>
<td>10.2 ± 2.9</td>
<td>9.7 ± 1.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 8. Values in a row bearing different letters are significantly different by Tukey’s test. A P value of 0.05 or less was considered to be significant.
2 Nutritional analysis was performed with Nutritionist IV version 2.0 (N-SQUARED COMPUTING, Salem, OR).

DISCUSSION

In Expt. 1, although the amount of fat and protein intake from self-selected or ad libitum diets was about 40% greater (P < 0.05) than that from the basic foods diet (Table 1), diet selection did not affect isoflavone bioavailability as seen in urinary recovery of ingested isoflavones (Table 2) or plasma isoflavone concentrations. Diet selection did not affect inter-individual variability in short-term isoflavone bioavailability because SD were similar across treatments (Table 2). The β-glucosidase and β-glucuronidase activities of cecal bacteria in rats were increased by increasing dietary protein intake from 0 to 40% of the diet for 10 d (Wise et al. 1983). Rats fed 35% beef fat or olive oil for 30 d had significantly lower total cecal bacterial β-glucosidase activity and greater total cecal bacterial β-glucuronidase activity by twofold in comparison to rats fed 1% safflower oil (Mallett et al. 1984). Metabolism of isoflavones by human gut bacteria may be important for isoflavone absorption and excretion (Xu et al. 1995). Bacterial β-glucosidases may be needed for isoflavone absorption with β-glucuronidase and sulfatase activities needed for isoflavone reabsorption after biliary excretion. From Expt. 1, absorption, excretion and plasma concentration of isoflavones while consuming soy-containing meals did not depend upon diets differing modestly in amounts of fat and protein. Ad libitum diets may be appropriate in short-term isoflavone feeding studies in humans, at least in the case of a fairly homogeneous subject population. In relatively long-term soy feeding studies, dietary fat and protein intake might affect soy isoflavone bioavailability through their effects upon gut bacterial biotransformation of isoflavones.

On average, cooked soybean, TVP and soy milk powder contain > 95% of total isoflavones as glucosides, whereas tofu contains about 20% of its isoflavones as aglycones, and in tempeh, 40% of isoflavones are aglycones (Wang and Murphy 1994). Glucosides of isoflavones may be poorly hydrolyzed by mamm-

TABLE 3

| Isoflavone excretion and recovery as percentage of ingested dose after feeding soy food types: cooked soybeans, texturized vegetable protein, tofu or tempeh
<table>
<thead>
<tr>
<th>Cooked soybean</th>
<th>Texturized vegetable protein</th>
<th>Tofu</th>
<th>Tempeh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein, total mg</td>
<td>20 ± 2</td>
<td>28 ± 4</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>Genistein, total mg</td>
<td>24 ± 2</td>
<td>32 ± 4</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>Total urinary excretion, mg</td>
<td>12 ± 6</td>
<td>18 ± 6</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Daidzein recovery, %</td>
<td>45 ± 16*</td>
<td>51 ± 10*</td>
<td>50 ± 10*</td>
</tr>
<tr>
<td>Genistein recovery, %</td>
<td>13 ± 6</td>
<td>13 ± 8</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>Fecal excretion, mg</td>
<td>1 ± 1</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>Intake excreted in urine + feces, % total isoflavone</td>
<td>30 ± 12</td>
<td>33 ± 9</td>
<td>34 ± 7</td>
</tr>
</tbody>
</table>

1 Values are means ± sd.
2 Urine samples included urine collected during the first 24 h after dosing plus the first urination of d 2. Urinary excretion of daidzein as a percentage of ingested dose was significantly greater than genistein excretion for all soy foods (P < 0.01).
3 For daidzein or genistein, urinary recovery of isoflavone was not significantly affected by the type of soy food (P > 0.01).
4 Fecal samples included all feces excreted before and during fecal marker excretion.

TABLE 2

Urinary isoflavone recovery as a percentage of intake after consumption of different background diets

<table>
<thead>
<tr>
<th>Urinary excretion (total mg)</th>
<th>Basic foods</th>
<th>Self-selected</th>
<th>Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>39 ± 12</td>
<td>35 ± 10</td>
<td>35 ± 9</td>
<td></td>
</tr>
<tr>
<td>Daidzein recovery, %</td>
<td>27 ± 8*</td>
<td>26 ± 11*</td>
<td>26 ± 8*</td>
</tr>
<tr>
<td>Genistein recovery, %</td>
<td>20 ± 10</td>
<td>18 ± 9</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>Fecal excretion, total mg</td>
<td>4 ± 4</td>
<td>4 ± 3</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>% total isoflavone dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>excreted in urine + feces</td>
<td>27 ± 10</td>
<td>25 ± 9</td>
<td>25 ± 9</td>
</tr>
</tbody>
</table>

1 Values are means ± SD; n = 8.
2 Urine samples included urine collected for 48 h after dosing plus the first urination of d 2.
3 The percentage of recovery was relative to the intakes of genistein (88 ± 16 mg/d) and daidzein (69 ± 12 mg/d). Urinary recovery of daidzein was significantly greater than that of genistein for all diets (P < 0.05).
4 Fecal samples included all feces excreted before and during dye marker excretion.
Ingested isoflavones are more hydrophilic than their aglycones, and their greater molecular weight should also limit their absorption (Brown 1988). Urinary isoflavones in both studies were 20–35% of ingested doses (Tables 2, 3), which were much greater than the percentage of aglycones in total isoflavones of cooked soybean, TVP or soy milk powder. It seems likely that hydrolysis of isoflavone glucosides to their aglycones by \( \beta \)-glucosidases of gut microflora or foods is needed for soy isoflavone absorption (Xu et al. 1995).

Although the four soy foods studied contain various amounts of isoflavone glucosides and aglycones, urinary recovery of either D or O was not significantly different among four soy food treatments (Table 3). These data suggest that difference in type of soy food does not affect isoflavone absorption and bioavailability, and gut bacterial \( \beta \)-glucosidases may have similar efficiency of hydrolysis for the various isoflavone glucosides. Similarly, almond \( \beta \)-glucosidases can hydrolyze the \( \beta \)-glucosidic bond between the carbohydrate moiety and the isoflavone nucleus regardless of the differences in carbohydrate group (Farmakalidis and Murphy 1985). The \( \beta \)-glucosidases in the human gut may act similarly to almond \( \beta \)-glucosidase.

Four soybean foods provided similar human bioavailability of isoflavonoids. Although tempeh may have had somewhat lesser bioavailability than did tofu, soy beans or TVP, in terms of total isoflavones recovered from the dose given, the small number of subjects did not permit the detection of any significant difference among the soy foods studied. There is great interindividual variability in isoflavone bioavailability (Tables 2 and 3) that such differences might not be easy to detect. The average recovery of isoflavones (% of dose excreted) from the four soy foods tested was about 30% (Table 3). After feeding single meals (0.9 mg isoflavones/kg body weight) of tofu and TVP to women, total isoflavone recovery was the same with either soy food, ~37% of the dose given (Tew et al. 1996). Recovery of isoflavones after soy milk feeding was about 26% (Table 2). In previous studies, recovery of isoflavones from soy milk was 17% (Xu et al. 1994) at each of three single doses (0.7–2.0 mg isoflavones/kg body weight) fed to women, and 14 or 34% in women fed three soy milk-containing meals during 1 d (Xu et al. 1995). The difference in isoflavone recovery between the two subgroups of women studied was that women who excreted 10-fold greater amounts of fecal isoflavones excreted more than twofold more urinary isoflavones. It seems that interindividual variation in isoflavone recovery may play a more important role than soy food form in determining human isoflavone bioavailability, but additional studies are needed.

Seemingly, it is only the total amount of isoflavones from the food, rather than the form of food that matters. The ratio of glycone/aglycone forms of isoflavones depends upon the processing of the different soy food forms, but this ratio may not be important to isoflavone bioavailability. A study comparing tofu and TVP (tofu usually contains a two-fivefold greater ratio of glycones/aglycones than did the soy beans) fed as part of all meals over 9 d to 17 men found 70% greater recovery of \( D \) when tempeh was fed than after feeding soy bean pieces. \( G \) was also recovered to a significantly greater extent after tempeh feeding than after consumption of soy bean pieces (Hutchins et al. 1995). In this study, the recovery data represent daily recoveries, rather than recovery of a single dose. The recovery of multiple doses fed during a day seems to be prolonged to about 48 h compared with 24 h for a single dose (see Xu et al. 1995 vs. Xu et al. 1994), but total recovery was comparable (14% when three doses of soy milk were given (Xu et al. 1995) vs. 17% when one soy milk dose was given (Xu et al. 1994). Therefore, study design probably does not account for the difference between the findings of Hutchins et al. (1995) and the present study. It is possible that the gender difference between the two studies accounts for the different results, but a gender difference in gut microfloral ability to metabolize glycosides seems unlikely.

Further studies to clarify longer-term influences on isoflavone bioavailability would be desirable, but short-term studies suggest, in general, that choice of background diet or soy food form would not be crucial determinants of the biological effects of soy isoflavones.

**LITERATURE CITED**


