A Diet High in Wheat Fiber Decreases the Bioavailability of Soybean Isoflavones in a Single Meal Fed to Women

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ABSTRACT The absorption of some dietary components may be inhibited by dietary fiber. To study the effect of dietary fiber on the bioavailability of isoflavones, seven healthy women were randomly assigned in a crossover design to a control diet containing 15 g dietary fiber or a wheat fiber—supplemented diet containing 40 g dietary fiber, both fed with a single dose of 0.9 mg isoflavones/kg body weight from tofu or texturized vegetable protein (TVP). The fiber-rich diet produced 55% lower plasma genistein at 24 h after soy dosing (P < 0.05) and reduced total urinary genistein by 20% (P < 0.03). Urinary daidzein was not significantly related to fiber intake. Highly insoluble, dietary wheat fiber reduced the absorption of genistein probably by its binding effect and hydrophobic binding to this compound. Urinary genistein was greater by 23% after tofu than after TVP consumption (P < 0.02), but the percentage of ingested genistein recovered in urine was not affected by soy product intake. The higher urinary genistein after tofu consumption compared with TVP was apparently due to differences in amount of genistein between these soy foods, not the different forms of genistein present in these two soy food products. J. Nutr. 126: 871–877, 1996.

INDEXING KEY WORDS:
• isoflavones • dietary fiber • bioavailability • humans

Lower mortality from hormone-dependent cancer is observed in populations excreting greater amounts of urinary flavones and isoflavones [Adlercreutz et al. 1982, 1986 and 1991]. A high urinary excretion of isoflavones correlates with the consumption of soy products (r = 0.585, P < 0.01) [Adlercreutz et al. 1991]. Soybean isoflavones make up 0.1–0.3 g/100 g of soybeans [Wang and Murphy 1994a]. Daidzein, genistein and their glycosides are the major isoflavones in soybeans [Murphy 1982] in which glycosides compose 97–98% of total isoflavones [Wang and Murphy 1994b]. Because of the structural similarity of isoflavones to estrogens, isoflavones can bind to estrogen receptors and exert estrogenic and antiestrogenic activities [Adlercreutz et al. 1982, Bannwart et al. 1984, Satchell et al. 1984, Tang and Adams 1977 and 1980]. Isoflavones reduce the biological activity and the level of estrogens by stimulating the production of plasma sex hormone binding globulin and inhibiting human aromatases [Adlercreutz et al. 1992a and 1993]. These effects of isoflavones may contribute to the prevention of sex-hormone–dependent cancers. Other activities of isoflavones may also contribute to their suggested anticancer effects. The antioxidant activity of isoflavones is related to the numbers of hydroxyl groups in the ring [Naim et al. 1976]. Isoflavones may prevent oxidative damage in the body by inhibiting the formation and scavenging of free radicals [Wei et al. 1993] and thus may contribute to anticarcinogenesis. Genistein, which can inhibit the tyrosine kinase activity of the epidermal growth factor, inhibits cell proliferation and neoplastic transformation [Akiyama et al. 1987].

A high fat, low fiber diet is thought to contribute to hormone-dependent neoplasia. Caucasian women who consume more fat and less dietary fiber than Asian women also excrete fewer fecal estrogens and have higher urinary estrogens [Goldin et al. 1986]. A high intake of dietary fiber in Asian women increases fecal weight, which positively correlates with fecal estrogens. The increase of fecal estrogens is negatively associated with urinary and plasma estrogens [Goldin et al. 1991].

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The type of dietary fiber seems important in affecting plasma estrogens. Finnish women with low breast cancer risk have higher grain fiber consumption than American women, but the total fiber intake of the two populations is the same. This difference in type of dietary fiber leads to greater fecal weight in the Finnish women compared with Bostonian women (Adlercreutz et al. 1992b). Large fecal bulk interferes with the enterohepatic circulation of sex hormones and increases fecal estrogen by decreasing the concentration of $\beta$-glucuronidases in the intestine (Goldin et al. 1982). Furthermore, insoluble fibers can bind to nonpolar estrogens (Whitten and Shultz 1988).

A series of determinations of the bioavailability of isoflavones were performed in our laboratory to establish the amounts of dietary isoflavone required to exert biological effects. Structurally the isoflavones are similar to estrogenic steroids. Because dietary fibers, especially insoluble fibers, can affect the metabolism of estrogens, similar effects may occur with isoflavones. Thus, the effect of dietary wheat bran on the bioavailability of isoflavones was investigated.

SUBJECTS AND METHODS

Study design. Two soy products, tofu and texturized vegetable protein (TVP), were added to the breakfast of controlled diets with and without fiber supplements to make up four dietary treatments. These four treatments were randomly assigned to female subjects on four different dosing days in a crossover design in which subjects and feeding days were blocked. There was a 2-wk washout period between soy food dosages to eliminate any carry-over effect from previous feedings.

Subjects. Seven healthy female subjects were 29.4 ± 9.7 y old (mean ± sd), with body weight of 61.2 ± 7.6 kg and body mass index (BMI) of 21.9 ± 2.26 kg/m$^2$ (mean ± SD). They were staff or students of Iowa State University (ISU). None of them were allergic to soy foods or ate soy products more than once a week. The procedures for this study were approved by the Human Subject Committee of ISU and U.S. Food and Drug Administration. The participation of each subject was approved by a physician at the Student Health Center at ISU. The informed consent of each subject was obtained in writing.

Treatments. Two soy products, tofu and TVP, were chosen as sources of isoflavones. Tofu was a gift from Dr. Lester Wilson, Food Science and Human Nutrition, ISU; TVP was purchased from Now Foods (Glendale Heights, IL). The extraction of isoflavones from these soy products was based on the method described by Wang et al. (1990). Total isoflavones were measured as free isoflavones after hydrolysis in 1 mol/L HCl. Isoflavone extract was analyzed by HPLC as described below. The total concentration of isoflavones (daidzein, genistein and their glycones) was 0.286 mg/g of tofu and 2.11 mg/g of TVP. The glycine content of these soy products was negligible. The composition of isoflavones in tofu differed from TVP. Genistein and its glycone (genistein equivalents) constituted 62.0% (0.18 mg/g) and 53.6% (1.1 mg/g) of isoflavones in tofu and TVP, respectively, whereas daidzein and its glycone (daidzein equivalents) constituted 38.0% (0.11 mg/g) and 46.4% (1.0 mg/g) of isoflavones in tofu and TVP, respectively.

Each subject consumed 0.9 mg of total isoflavones per kilogram of body weight from tofu or TVP regardless of the composition of daidzein and genistein forms in the soy foods. High and low fiber diets were designed to meet the energy and nutritional needs of subjects. These diets were nearly the same except for fiber supplementation of the fiber-rich diet. Wheat bran cereal (Allbran, Kellogg, Battle Creek, MI) and whole wheat bread instead of white bread were given in the fiber-rich diet. There were about 40 g dietary fiber in the fiber-rich diet and 15 g dietary fiber in the regular diet. Insoluble fiber constituted most of the dietary fiber in the fiber-rich diet (Table 1). Furthermore, tofu and TVP combined with these two diets contributed to slightly different nutritional and energy contents of the diets. Fiber contribution from the soy sources was negligible. Fat content was nearly 30% of total energy in all four diets. The composition of the diets was analyzed by Nutritionist IV (N-Squared Computing, Salem, OR) (Table 1).

Diet preparation. Specific amounts of tofu or TVP for each subject were weighed and mixed with egg, sharp cheddar cheese and salsa to make microwavable casseroles; ground wheat bran cereal was also added to make casseroles for the fiber-rich diet. Lunch and din-

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wheat fiber-supplemented diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tofu</strong></td>
<td>11.16</td>
<td>10.70</td>
</tr>
<tr>
<td><strong>TVP</strong></td>
<td>10.29</td>
<td>9.84</td>
</tr>
<tr>
<td><strong>Energy, MJ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fat, en%</strong></td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td><strong>Protein, en%</strong></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Carbohydrate, en%</strong></td>
<td>56</td>
<td>55</td>
</tr>
<tr>
<td><strong>Dietary fiber, g</strong></td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td><strong>Insoluble fiber, g</strong></td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td><strong>Soluble fiber, g</strong></td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Percentage of energy intake. All diets were otherwise nutritionally adequate, containing at least 90% of the recommended dietary intake (RDI) for each nutrient.
ner sandwiches were made with bread and cheese. In the high fiber diet, ground wheat bran cereal was added to yogurt.

A 1-g carmine red fecal marker (Pharmaceutical Service, University of Iowa, Iowa City, IA) was taken at breakfast. Lunch, supper and snack were supplied for each day of soy feeding.

**Sample collection. Blood samples.** Blood was drawn half an hour before, and 6.5 h and 24 h after soy product consumption in the breakfast. After collection, blood samples were centrifuged at 3000 × g for 10 min, then plasma was separated from blood and stored at −20°C before analysis.

**Urine collection.** Urine samples were collected before soy product consumption, over 24 h after dosing and the first urination later than 24 h after dosing. All of the samples were grouped into four periods of time, before dosing, first 12 h, second 12 h and the first urination after 24 h. Then, 50 mL aliquots of urine were stored in a −20°C freezer until analysis.

**Fecal samples.** One fecal sample was collected before dosing and feces were collected after dosing as indicated by the fecal marker. Then, feces were freeze-dried, weighed and ground into powder. All of the fecal samples collected after dosing were mixed to homogeneity, then 10 g of ground feces as stored in vials kept at 4°C.

**Sample analysis.** The analytical methods developed for urinary and plasma samples were adapted from the determination of daidzein and equol in bovine blood plasma and urine in Lundh et al. [1988]. Plasma (1 mL) and urine (2.5 mL) were incubated with β-glucuronidases (Sigma Chemical, St. Louis, MO) at 37°C for 16 h to release aglycones of isoflavones. Separation of isoflavone from soybean products as outlined in Murphy [1981 and 1982] was modified to extract the isoflavones from feces. Ground feces (2 g) were treated with 10 mL acetonitrile and 2 mL 0.1 mol/L HCl (Xu et al. 1994). The extract was dissolved in methanol:water (4:1) for HPLC analysis.

**HPLC analysis.** Plasma, urine and fecal isoflavones were separated by C18 reverse-phase column (10 μm, 3.9 mm i.d. × 30 cm length, Waters, Milford, MA) with gradient elution at ambient temperature. Two mobile phases, HPLC grade water and methanol, produced a gradient of 40–80% methanol within 40 min. Samples were injected by autosampler model 8780 XR (Spectra-Physics, Fremont, CA). Peaks were detected by UV absorbance (model 163 variable wavelength detector, Beckman, Fullerton, CA) at 254 nm and 0.2 absorbance units full scale (AUFS) sensitivity. The peak area was measured by a model 427 integrator (Beckman). The identification of daidzein and genistein was done by comparison with the retention time of the corresponding standards. Standard curves for daidzein and genistein were established with a series of concentrations of each standard to quantify extracted daidzein and genistein. Recoveries of isoflavones from plasma, urine and feces were obtained by spiking some of those samples with known concentrations of daidzein (ICN Biochemical, Costa Mesa, CA) and genistein (Sigma Chemical).

**Statistical analysis.** ANOVA was performed on all data collected in this factorial, cross-over design study. Effects of four treatments were analyzed by General Linear Models (GLM) on the data from blood, urine and feces. Plasma concentrations of daidzein and genistein at certain time points were analyzed separately and by repeated measurement analysis. Urinary excretion of daidzein and genistein during the first and second 12 h after dosing, the first urination later than 24 h after dosing and their total was analyzed separately by GLM. Amounts of daidzein and genistein in soy products, plasma, urine, feces were compared by t test. A P value of 0.05 or less was considered to be significant. The statistical analysis was performed by Statistical Analysis System (Cary, NC) version 6.06 on the Iowa State University mainframe computer.

**RESULTS**

**Isoflavone content in tofu and TVP.** Because tofu and TVP had different isoflavone compositions, the amount of daidzein and genistein equivalents ingested differed with soy foods (P < 0.0002 and P < 0.004, respectively). A 21.0 ± 2.5 mg daidzein (mean ± SD) and 34.2 ± 4.1 mg genistein equivalent were consumed from tofu whereas a 25.6 ± 3.1 mg daidzein and 29.6 ± 3.6 mg genistein equivalent were consumed from TVP. The ratio of daidzein equivalent to genistein equivalent [D:G] differed significantly between tofu [0.61] and TVP [0.86] (P < 0.0001).

**Plasma.** Cumulative plasma concentrations of daidzein and genistein were not altered by dietary fiber or soy products. Plasma concentration of daidzein and genistein did not differ with treatments at 6.5 h after dosing. At 24 h after dosing, the high fiber diet produced 50% lower plasma daidzein concentration than did the low fiber diet (P < 0.08). After consumption of a fiber-rich diet, plasma genistein was significantly less than after consumption of the diet lower in fiber (P < 0.05) [Table 2].

**Urine.** Higher total urinary genistein was found after feeding a low fiber diet (19.2 ± 10.0 μmol) compared with a fiber rich diet (15.5 ± 10.0 μmol) (P < 0.03) [Table 3]. Tofu consumption produced 19.6 ± 10.0 μmol urinary genistein, which was higher than after TVP consumption (15.2 ± 10.2 μmol) (P < 0.02). Urinary genistein in the first 12 h after soy feeding was greater after tofu feeding than after TVP feeding, whereas at the first urination after 24 h, urinary genistein was greater after TVP than after tofu feeding (P < 0.02 and P < 0.04, respectively) [Table 3]. All treatments caused similar urinary excretion of daidzein (data not shown). When daidzein and genistein were
TABLE 2
Plasma concentration of daidzein and genistein at 6.5 h and 24 h in women consuming wheat fiber–supplemented or control diets containing tofu or texturized vegetable protein (TVP)1

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>n</th>
<th>6.5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Daidzein</td>
<td>Genistein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μmol/L</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Overall</td>
<td>28</td>
<td>1.44 ± 0.54</td>
<td>1.33 ± 0.43</td>
</tr>
<tr>
<td>Wheat fiber</td>
<td>14</td>
<td>1.32 ± 0.53</td>
<td>1.30 ± 0.47</td>
</tr>
<tr>
<td>Control diet</td>
<td>14</td>
<td>1.57 ± 0.54</td>
<td>1.35 ± 0.40</td>
</tr>
<tr>
<td>Tofu</td>
<td>14</td>
<td>1.43 ± 0.64</td>
<td>1.36 ± 0.49</td>
</tr>
<tr>
<td>TVP</td>
<td>14</td>
<td>1.46 ± 0.45</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>Wheat fiber, tofu</td>
<td>7</td>
<td>1.38 ± 0.70</td>
<td>1.39 ± 0.64</td>
</tr>
<tr>
<td>Control diet, tofu</td>
<td>7</td>
<td>1.47 ± 0.62</td>
<td>1.33 ± 0.35</td>
</tr>
<tr>
<td>Wheat fiber, TVP</td>
<td>7</td>
<td>1.25 ± 0.32</td>
<td>1.20 ± 0.23</td>
</tr>
<tr>
<td>Control diet, TVP</td>
<td>7</td>
<td>1.68 ± 0.47</td>
<td>1.38 ± 0.48</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 Plasma concentration of genistein at 24 h was significantly lower from subjects consuming wheat fiber–supplemented diet compared with control diet (P < 0.05).

combined, total isoflavone excretion was lower after feeding a fiber-rich diet, 57.8 ± 20.0 μmol compared with a low fiber diet, 66.6 ± 18.1 μmol (P < 0.05) but was not related to soy product consumption. Excretion of daidzein and genistein was greater within the first 12 h than in the second 12 h after dosing. The isoflavones were almost totally excreted from the body at 24 h after dosing. Urinary daidzein was much greater than urinary genistein during each time interval (P < 0.0001). The ratio of daidzein to genistein (D:G) in urine was affected by soy products (P < 0.01). Urinary D:G was 2.7 ± 1.7 after tofu feeding and 4.1 ± 2.0 after TVP consumption.

Feces. Fecal daidzein, genistein and total isoflavones were the same for all treatments. The mean excretion of fecal daidzein and genistein was 3.5 ± 2.3 μmol and 3.1 ± 2.3 μmol (mean ± SD), respectively.

The proportion of the ingested isoflavones excreted. Total urinary daidzein excretion was 49 ± 11% of total ingested daidzein, which apparently was not affected by any treatment. The recovery of urinary genistein from ingested genistein was lower with high dietary fiber (P < 0.03) but did not vary with the type of soy product consumed. Genistein recovery was 13 ± 8% after feeding a fiber-rich diet and 16 ± 8% after feeding a diet containing less fiber. Lower urinary

TABLE 3
Urinary excretion of genistein over time by women fed wheat fiber–supplemented or control diets containing tofu or texturized vegetable protein (TVP)1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Time interval after dosing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time 0</td>
<td>0–12 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μmol</td>
<td>μmol</td>
</tr>
<tr>
<td>Overall</td>
<td>28</td>
<td>ND</td>
<td>9.62 ± 5.55</td>
</tr>
<tr>
<td>Wheat fiber</td>
<td>14</td>
<td>ND</td>
<td>8.88 ± 5.78</td>
</tr>
<tr>
<td>Control diet</td>
<td>14</td>
<td>ND</td>
<td>10.73 ± 5.55</td>
</tr>
<tr>
<td>Tofu</td>
<td>14</td>
<td>ND</td>
<td>11.47 ± 5.92</td>
</tr>
<tr>
<td>TVP</td>
<td>14</td>
<td>ND</td>
<td>8.14 ± 4.07</td>
</tr>
<tr>
<td>Wheat fiber, tofu</td>
<td>7</td>
<td>ND</td>
<td>10.73 ± 5.18</td>
</tr>
<tr>
<td>Wheat fiber, TVP</td>
<td>7</td>
<td>ND</td>
<td>7.03 ± 4.81</td>
</tr>
<tr>
<td>Control diet, tofu</td>
<td>7</td>
<td>ND</td>
<td>12.21 ± 7.40</td>
</tr>
<tr>
<td>Control diet, TVP</td>
<td>7</td>
<td>ND</td>
<td>8.89 ± 3.33</td>
</tr>
</tbody>
</table>

1 Mean ± SD. ND = nondetectable.
2 Total genistein in wheat fiber–supplemented diet was significantly less than in control diet (P < 0.03).
3 Urinary genistein from tofu was significantly greater than that from TVP during first 12 h after dosing (P < 0.02).
4 Urinary genistein from tofu was less than that from TVP at the first urination after 24 h dosing (P < 0.04).
5 Total urinary genistein was higher after tofu consumption than after TVP consumption (P < 0.02).
isoflavones were recovered after feeding a high fiber diet (27 ± 9%) than after feeding a diet lower in fiber (31 ± 8%) (P < 0.05). There was a significant difference between the recoveries of urinary daidzein and genistein (P < 0.0001). Fecal daidzein and genistein excretion, as percentages of their ingested amounts, were 3.7 ± 2.3 and 2.5 ± 2.0%, respectively. If excretion of daidzein and genistein in urine and feces were considered together, the total daidzein excreted was 53 ± 11% of ingested daidzein whereas the total genistein excreted was 17 ± 9% of ingested genistein.

**DISCUSSION**

In our study, about 15 g dietary fiber was present in a control diet, whereas a fiber-rich diet was supplemented with wheat bran cereal and wheat bread substituted for white bread to provide about 40 g dietary fiber without any reduction of fat intake during the dosing day. Because we fed an amount of fiber similar to that fed by Rose et al. (1991), we expected a suppression of isoflavone bioavailability similar to what Rose et al. observed for estrogens, which are structurally similar to isoflavones. Rose et al. (1991) doubled fiber intake of 62 premenopausal women from about 15 g to 30 g with wheat, oat, or corn bran. After 2 mo, the dietary fiber supplementation with wheat bran significantly reduced serum estrone (P < 0.002) and estradiol (P < 0.02) by 15–20%, but oat and corn bran supplement did not affect serum estrogens. Estrogens are conjugated with glucuronic acid in liver, then 20–80% of conjugated estrogens are excreted in bile. The reabsorption of estrogens requires the hydrolysis of β-glucuronidases, releasing free form estrogens. Wheat bran may reduce the activity of β-glucuronidases and the reabsorption of estrogens, thus contributing to fewer serum estrogens. Furthermore, wheat bran fibers may also bind to unconjugated estrogens in the gut, interfering with estrogen reabsorption. In our study, total urinary excretion of genistein was significantly decreased by 20% when a fiber-rich diet was fed compared with the control diet (P < 0.03) [Table 3]. Even though urinary daidzein was not significantly affected by fiber intake, the total urinary excretion of isoflavones (daidzein and genistein) was reduced 13% by increased fiber intake (P < 0.05). This result supported our previous assumption that wheat bran fiber may suppress isoflavone absorption just as it suppresses estrogen absorption.

The ability of dietary fibers from different sources to bind with conjugated and unconjugated estrogens in vitro has been measured (Shultz and Howie 1986). Of dietary fibers, wheat had the higher percentage of estrogen binding, followed by oat and corn. Conjugated estrogens were more weakly bound by each type of fiber. Enhanced adsorption of the less hydrophilic, mono- and dihydroxy-steroids was observed on a diet high in dietary fiber. About 70% of estrone and 17β-estradiol bound to water-insoluble fibers in vitro (Whitten and Shultz 1988). Therefore, insoluble dietary fibers from wheat fiber may bind to isoflavones, especially free isoflavones, impeding their absorption throughout the gut. Urinary excretion of genistein was affected by fiber intake but urinary daidzein was not, probably because genistein is more hydrophobic than daidzein (Satchell and Welsh 1987). This is consistent with the preference for hydrophobic binding between dietary fibers and estrogens observed by Shultz and Howie (1986) and the theory of the hydrophobic nature of binding of vegetable fibrous tissue with bilt salts and acids, which are somewhat structurally similar to estrogens and isoflavones (Eastwood and Hamilton 1968).

The presence of insoluble fibers and their capacity to hold water also cause bulking of the luminal contents of the intestine. The bulking in the gut may dilute the concentration, and thus, activity of gut microflora. Mallett et al. (1983) measured the effect of cellulose on the activity of rat cecal microflora. When 0–40% cellulose was added to the purified diet, the total numbers of cecal microflora were decreased in a concentration-dependent manner, corresponding to the increase of the luminal contents. When dietary cellulose was 10% or more, β-glycosidase activity was decreased, whereas β-glucuronidase activity was reduced by 40% dietary cellulose. Wheat bran supplementation caused a reduction of fecal β-glucuronidase activity, whereas corn bran exerted no significant effect on this enzyme (Rose et al. 1991). Almost 97–98% of isoflavones are glycosides in soybeans (Wang and Murphy 1994b). Rowland (1991) suggested that plant glycosides are poorly absorbed because of their polarity and that they cannot be hydrolyzed by digestive enzymes. Friend and Chang (1984 and 1985) studied the hydrolysis of 21-yl-β-D-glucosides and galactosides of dexamethasone, prednisolone, hydrocortisone and fluorocortisone, and 21-yl-β-D-cellobiosides of prednisolone in rat stomach, proximal small intestine, distal small intestine and cecum. All of the glycosides were slowly hydrolyzed by stomach and proximal intestine, more rapidly by distal intestine and most rapidly in the cecum. When dexamethasone and prednisolone were administered to rats, these aglycones were absorbed almost completely from the small intestine. Because humans have a relatively low bacterial population in the stomach and small intestine and a bacterial population in the large intestine comparable with the rat, gut microflora in large intestine may alter the absorption and reabsorption of isoflavones. Therefore, insoluble fibers which can dilute the concentration of gut microflora might decrease the hydrolysis of isoflavones and reduce their absorption and reabsorption. How likely it is that the hydrolysis of isoflavones will occur in the human stomach [pH 1–3] is unknown. Wang et al. (1990) found that the most favorable conditions for the hydrolysis of isoflavone glycosides in soybeans and processed soy products
were 1 mol/L HCl for 2 h at 98–100°C. These conditions are more harsh than those found in the stomach. Although most urinary isoflavone excretion occurs in the first 12 h after dosing, the absorbable free isoflavones are most likely produced in the lower small intestine and large intestine by the action of gut microorganisms.

Total urinary excretion of genistein was related to the consumption of soy products \( P < 0.02 \) (Table 3) but urinary daidzein was not. Total genistein excretion in urine was significantly greater after tofu ingestion. This was consistent with higher total genistein consumption from tofu and higher total daidzein consumption from TVP. Urinary D:G after tofu consumption \( (2.7 \pm 1.7) \) compared with TVP consumption \( (4.1 \pm 2.0) \) corresponded with the D:G ratio in soy products, 0.61 in tofu and 0.86 in TVP. The distribution of isoflavone forms in commercial soy food products is influenced by processing, but whether different isoflavone forms affect the absorption of daidzein and genistein is unknown. TVP usually contains large amounts of daidzin and genistin, and moderate 6"-O-acetyldaidzin and 6"-O-acetylgensitin, whereas tofu has much more 6"-O-malonyldaizin and 6"-O-malonylgenistin than does TVP (Wang and Murphy 1994b). The proportion of total genistein ingested to urinary genistein was not affected by soy product intake, indicating that the soy product effect was due only to the different amounts of genistein found in the two soy foods, and not to the different isoflavone forms present in the soy products. Almond β-glycosidases hydrolyze the β-glycosidic bond between the carbohydrate moiety and the isoflavonoid nucleus regardless of any difference in carbohydrate group (Farmakalidis and Murphy 1985). Differences in isoflavone forms may not affect isoflavone hydrolysis if gut β-glycosidases function similarly to almond β-glycosidases, and thus the ingested form would not affect isoflavone absorption. A companion study, which investigated the effect of fat on the bioavailability of isoflavones from the same soy products, tofu and TVP, showed similar results (Tew, B. Y., Wang, H. J., Murphy, P. A., and Hendrich, S., unpublished observations). In that study, although total urinary daidzein was greater after TVP consumption than after tofu intake, the proportion of ingested daidzein excreted in urine did not differ with type of soy product. The present study showed that urinary excretion of daidzein, \( 46.0 \pm 13.2 \mu mol \), was much greater than urinary excretion of genistein, \( 17.4 \pm 10.0 \mu mol \) \( P < 0.0001 \) (Table 3). The proportion of ingested daidzein excreted was greater than the proportion of ingested genistein excreted in urine \( P < 0.0001 \) (49 and 15%, respectively). Furthermore, D:G in urine was greater than the D:G in the soy products. These results indicated greater bioavailability of daidzein than genistein. A study of bioavailability of soybean isoflavones in women ingesting 0.7, 1.3 and 2.0 mg isoflavones/kg body weight as a single dose from soy milk found that 21% of total daidzein ingested and 9% of total genistein ingested appeared in urine (Xu et al. 1994). In a study of the effect of fat on the bioavailability of isoflavones (0.9 mg isoflavones/kg body wt consumed in a single dose from tofu and TVP), 30% of total daidzein ingested and 20% of total genistein ingested, appeared in urine (Tew, B. Y., Wang, H. J., Murphy, P. A., and Hendrich, S., unpublished observations). These studies indicate greater bioavailability of daidzein than genistein, although the extent of the recovery of urinary daidzein and genistein varied. Less genistein appeared in urine probably because more genistein was excreted into bile due to its higher molecular weight and hydrophobicity. The isoflavones in the lower gut are likely to be degraded by microflora (Xu et al. 1995). Only traces of daidzein and genistein were detected in feces. Gut microflora may degrade daidzein and genistein to unknown compounds, thus the recovery of daidzein and genistein in feces would be much less than ingested amounts. This has been shown in previous studies (Xu et al. 1994).

Most daidzein and genistein was excreted in urine within the first 12 h after dosing (65 and 60%, respectively). At the first urination 24 h after dosing, urinary daidzein and genistein decreased to 2.0 μmol and 0.74 μmol, respectively (about 5% of the total for both). Plasma daidzein and genistein had almost disappeared at 24 h after dosing, indicating no retention of isoflavones in the body. This was also consistent with previous studies. No urinary equol was found after a single isoflavone dose, indicating the requirement of the induction of microfloral enzymes which can convert daidzein to equol after repeated isoflavone doses (Setchell et al. 1984).

Insoluble fibers from wheat bran cereal affect the bioavailability of isoflavones probably by their ability to bind to isoflavones, impeding their absorption. The bulking effect of wheat bran may decrease the concentration of microfloral enzymes such as β-glycosidases and β-glucuronidases, limiting reabsorption of isoflavones excreted in bile. Therefore, lower urinary isoflavones were detected after feeding a fiber-rich diet. The urinary excretion of genistein, but not daidzein, was affected by increased wheat bran consumption, indicating the hydrophobic nature of binding between insoluble fibers and isoflavones. Urinary excretion of genistein differed with soy products due only to the difference in ingested amount of genistein and not to the different isoflavone forms present in tofu and TVP.

Because high fiber intake will reduce the absorption of isoflavones to some extent, such a diet may affect the biological function of isoflavones. In determining isoflavone consumption required to maintain a certain physiological level of isoflavones, the fiber content in the diet should be considered. What amounts and types of dietary fibers can affect the bioavailability of isoflavones should also be further investigated.
LITERATURE CITED


