Human Nutrition and Metabolism

Urinary Disposition of the Soybean Isoflavones Daidzein, Genistein and Glycitein Differs among Humans with Moderate Fecal Isoflavone Degradation Activity

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ABSTRACT Glycitein metabolism was compared with other isoflavones to begin to understand the effect of this compound. Total isoflavones of 4.5 \( \mu \)mol/kg body weight from soymilk (high in genistein and daidzein) and soygerm (high in daidzein and glycitein) was fed to seven women and seven men. To minimize interindividual variation, only subjects with moderate fecal isoflavone degradation rates (half-lives of daidzein and genistein were 15.7 and 8.9 h, respectively) were included. The average 48-h urinary excretion of glycitein, daidzein and genistein was 55, 46 and 29% of the dose ingested, respectively, which was significantly different from each other in men and women (\( P < 0.001 \)). Plasma isoflavone concentrations at 6 and 24 h after soymilk feeding paralleled relative amounts of isoflavones in soymilk (genistein > daidzein > glycitein) (\( P < 0.05 \)) in men and women, but plasma isoflavone concentrations after soygerm feeding did not parallel soygerm isoflavone concentrations in women because genistein and glycitein did not differ from each other at 6 h after feeding. Six hours after soygerm dosing, plasma isoflavone concentrations paralleled soygerm isoflavone levels in men. Based on plasma isoflavone concentrations at 6 h after dosing, the bioavailabilities of daidzein and genistein were similar in men and women. At the high glycitein dose (soygerm), plasma concentration at 24 h after dosing suggested a modest gender difference in glycitein bioavailability. J. Nutr. 129: 957–962, 1999.

Key Words: • fecal isoflavone degradation • glycitein • humans • isoflavones • urinary disposition

Isoflavones are one of the principle classes of plant-derived diphenols in the human food supply. Because the presence of isoflavones in human urine was related to lower mortality from sex hormone-dependent cancers, isoflavones have excited scientific researchers (Barnes and Messina 1991). Isoflavones might be partly responsible for soy's ability to lower the risk of cardiovascular diseases (Anderson et al. 1995) and to prevent bone mineral loss in ovariectomized rats (Arjmandi et al. 1996). Daidzein, genistein and their corresponding glucosides account for the major portion of soy isoflavones and have been the focus of numerous studies. But a third soy isoflavone, glycitein, may also contribute to soy's health effects.

Systemic studies of the absorption, metabolism and excretion of isoflavones are needed to determine their bioavailabilities and biological effects. Among the soy isoflavones, a greater proportion of the dose of daidzein was excreted in urine than that of genistein (King and Bursill 1998, Watanabe et al. 1998 Xu et al. 1994). Watanabe et al. (1998) reported maximum plasma concentrations of 2.44 ± 0.65 \( \mu \)mol/L at 6 h for genistein and 1.56 ± 0.34 \( \mu \)mol/L at the same time for daidzein after ingestion of 103 \( \mu \)mol daidzein and 112 \( \mu \)mol genistein, respectively. King and Bursill (1998) reported a peak of 4.09 ± 0.94 \( \mu \)mol/L at 8.42 ± 0.69 h for genistein and 3.14 ± 0.36 \( \mu \)mol/L at 7.42 ± 0.74 h for daidzein after 3.6 \( \mu \)mol genistein and 2.7 \( \mu \)mol daidzein/kg body weight soy meal ingestion, respectively. The plasma concentration versus time curves of daidzein and genistein were the same. Daidzein and genistein may have similar bioavailabilities, but the longer elimination half-life of genistein may contribute to its potential for greater efficacy than daidzein.

Glycitein and its corresponding glucosides account for 5–10% of the total isoflavones in most soy foods. In soymilk, glycitein accounts for at least 40% of total isoflavones. No biological activity or bioavailability studies of glycitein have been reported to date. Therefore, it is of great interest to study the bioavailability of glycitein, which may be an important determinant of glycitein's biological potency.

Gut microflora may play an important role in isoflavone degradation and may be a critical factor in determining isoflavone bioavailability. Xu et al. (1995) showed that two women...
who excreted greater amounts of fecal isoflavones had greater urinary and plasma isoflavone levels than five other women who excreted small amounts of isoflavones in feces, and these high excretors experienced more prolonged daidzein and genistein bioavailability. Gut motility and gut microflora differ among individuals. A study of gut microfloral metabolism of isoflavones in vitro in 15 subjects over a 10-mo period showed that the subjects sorted into three distinguishable groups with respect to the ability of their feces to degrade daidzein and genistein. Degradation rate constants for daidzein and genistein (kD and kG, respectively) (calculated as the negative slope of the regression line plotted for isoflavone content as a function of incubation time intervals. The equation used was ln(Co/C) = kt, with Co represented the initial isoflavone concentration and k represented the rate constant; t was the reaction time. The time needed for half of the isoflavone to disappear (t 1/2 ) from the culture medium was derived from the equation t 1/2 = ln(2)/k.)

The logarithm of isoflavone concentration (C) was plotted against incubation time intervals. The equation used was ln(Co/C) = kt, with Co represented the initial isoflavone concentration and k represented the rate constant; t was the reaction time. The time needed for half of the isoflavone to disappear (t 1/2 ) from the culture medium was derived from the equation t 1/2 = ln(2)/k. Half-lives of daidzein and genistein for participants were calculated (Table 1).

### Experimental procedures

**Subject screening.** The participants in this experiment were selected from 25 volunteers according to their gut microfloral ability to degrade isoflavones. Freshly voided fecal samples from volunteers were diluted and homogenized with sterilized brain-heart infusion culture medium (DIFCO Laboratories, Detroit, MI), containing 0.5 mol cysteine hydrochloride (Aldrich Chemical, Milwaukee, WI) as a reducing agent and 1 mg resazurine (Aldrich Chemical) as an indicator, under anaerobic conditions. After centrifugation, supernatant was added to duplicate sterilized culture tubes containing sterilized daidzein and genistein (final concentration of each compound 590 \( \mu \)mol/L) and incubated anaerobically at 37°C. At time 0, 6, 12, 24, and 48 h, 3 mL of culture mixture was obtained and mixed with 0.6 mL of 100% methanol and 0.9 mL of 0.4 mol trichloroacetic acid/L (Sigma, St. Louis, MO) containing 0.6 mol glycine/L (pH 2.0).Centrifuged and filtered daidzein and genistein were analyzed by HPLC, as described below.

The logarithm of isoflavone concentration (C) was plotted against incubation time intervals. The equation used was ln(Co/C) = kt, with Co represented the initial isoflavone concentration and k represented the rate constant; t was the reaction time. The time needed for half of the isoflavone to disappear (t 1/2 ) from the culture medium was derived from the equation t 1/2 = ln(2)/k. Half-lives of daidzein and genistein for participants were calculated (Table 1).

**Participants.** Fourteen healthy subjects (seven male and seven female), aged between 19 and 35 y, a body weight of 66.0 ± 11.2 kg, body mass index of 22.4 ± 2.3 kg/m², participated in this study (Table 1). The Human Subject Committee of Iowa State University (ISU) approved the procedures for this feeding study. Informed consent of subjects was obtained in writing.

**Diet.** The study consisted of two breakfast feedings, which were separated by a 1-wk washout period. Subjects were randomly assigned to consume either soymilk (Fearn Natural Foods, Mequon, WI) or soygerm powders (Soylife™, Schouten USA, Minneapolis, MN) mixed into cranberry or orange juice with a freely consumed breakfast chosen from wheat toast, cereals, skim milk, apple, banana, or orange in a crossover design. The total amount of soy isoflavones fed from...
Isoflavone concentration of soy isoflavone sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Daidzein (µmol/g)</th>
<th>Genistein (µmol/g)</th>
<th>Glycitein (µmol/g)</th>
<th>Total (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soymilk</td>
<td>3.9</td>
<td>4.4</td>
<td>0.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Soyge D</td>
<td>43.3</td>
<td>48.9</td>
<td>7.8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1 Sample analysis was performed in duplicate.

Either soy source was 4.5 µmol/kg body weight. All subjects were instructed to avoid any soy food and products containing texturized vegetable protein and hydrolyzed vegetables. A list of soy-containing food products is presented in the table.

**Blood sample collection.** Venous blood samples (10 mL) were collected into EDTA-containing vacuum containers by a licensed medical technologist under stringent aseptic conditions. A blood sample was collected 18 h before dosing (baseline), and at 6 and 24 h after soy-containing breakfast was consumed. Samples were centrifuged within 1 h after collection at 3000 g for 25 min at 4°C (Model 4D, International Equipment, Needham Hts., MA). Plasma was separated and stored in a -20°C freezer before analysis.

**Urine sample collection.** Each subject provided a urine sample immediately before dosing (time 0). After dosing, urine from each subject was pooled over the following time periods: 0–6, 6–12, 12–24, and 24–48 h. The total sample volume was recorded, and 50 mL aliquots of each pooled sample were stored in a -20°C freezer until analysis.

**Analysis methods.**

**Soy products analysis.** Two soy products, soymilk and soy germ powders, were chosen as glycine sources. The sample extraction and concentration determination were modified from the method described by Wang and Murphy (1994). Two grams of each soy product were extracted with 10 mL of acetonitrile and 2 mL of 0.1 mol HCl/L for 2 h at room temperature. The filtered and vaporized sample was dissolved in 10 mL of 80% methanol in water and analyzed by HPLC.

The total isoflavone content was the sum of total daidzein, total genistein, and total glycitein. Plasma and urine samples were analyzed for the isoflavones daidzein, genistein, and glycitein at certain time points were analyzed separately and by repeated measurement analysis. Urinary excretions of the three isoflavones during 0–12 h post dose were analyzed separately. Plasma samples were randomly spiked with daidzein, genistein, and glycitein standards to measure recoveries (0.1 g isoflavone/100 L plasma was added to each sample). Plasma and urine isoflavones obtained from feeding study were calculated with adjustment for recoveries.

**Statistical methods.** ANOVA was performed on the data obtained from this experiment with the SAS program (version 6.03, SAS Institute, 1995, Cary, NC). Plasma concentrations of daidzein, genistein, and glycitein at certain time points were analyzed separately and by repeated measurement analysis. Urinary excretions of the three isoflavones during 0–12 h post dose were analyzed with the urinary excretions from 12–48 h by paired t-test. Data from male and female subjects were analyzed separately for both plasma and urine. Tukey’s test was used for comparison within factors if there was a significant effect. A P value of 0.05 or less was considered to be significant. All values are reported as means ± SD.

**RESULTS**

Soymilk powder contained 9.0 µmol isoflavones/g—43.3% daidzein, 48.9% genistein, and 7.8% glycitein—whereas soy germ powder contained 71.4 µmol isoflavones/g—48.5% daidzein, 12.6% genistein and 38.9% glycitein (Table 2). Among the selected 14 subjects, the average fecal incubation half-lives of daidzein and genistein were 15.7 ± 5.3 and 8.9 ± 4.3 h, respectively (Table 1).

Urinary recoveries over the concentration range used for daidzein, genistein and glycitein were linear (R² = 0.9984, 0.9979, and 0.9928, respectively). The urinary recoveries of daidzein, genistein and glycitein were 76.4 ± 3.5, 85.6 ± 3.1, and 61.6 ± 4.2%, respectively. Recoveries of plasma isoflavones were daidzein, 76.5 ± 5.2%; genistein, 72.3 ± 4.8%; and glycitein, 63.6 ± 4.6%.

After soy milk feeding, the plasma glycitein concentrations in men and women were significantly lower than those of daidzein and genistein, and daidzein was significantly lower than genistein (P < 0.001) at both 6 and 24 h (Table 3). At 6 h after soy germ feeding, the plasma concentration of daidzein was significantly higher (P < 0.001) than genistein or glycitein in men and women (Table 3). Glycitein concentration was significantly (P < 0.001) higher than that of genistein in men but not in women. At 24 h after soy germ feeding, significantly different plasma concentrations of daidzein and genistein were only found in women with plasma genistein concentration intermediate.

The two soy treatments had no significant influence on the urinary isoflavone excretion as a percentage of ingested doses (Table 4). Men and women had similar urinary recoveries after consuming these two soy products, so pooled results are presented in the table.
excretion varied by as much as 8-fold (8.5–69.6%), daidzein by 5-fold (17.4–87.7%) and glycitein by 4.5-fold (19.7–91.3%), among the soy product treatments. Urinary excretion of daidzein and glycitein from both soy products and genistein from soymilk were significantly greater during the first 12 h than at later times (P< 0.05).

**DISCUSSION**

Gut microflora play important roles in isoflavone metabolism and bioavailability (Xu et al. 1995). It is possible that the interindividual variation of isoflavone bioavailability may be due partly to the action of gut microflora. To better characterize the bioavailability of isoflavones, in this study subjects were chosen from volunteers according to the ability of their fecal microflora to degrade isoflavones. The moderate fecal isoflavone metabolism rate phenotype was distinguished by the character that their fecal bacteria could rapidly degrade genistein with average half-lives (G1⁄2) of 5.0 h and daidzein (D 1⁄2) of 17 h (Wang 1997). In this study, the mean fecal degradation half-lives of genistein and daidzein were 8.9 and 15.7 h, respectively. Less fecal degradation would result in the appearance of greater amount of isoflavones in the circulation and greater urinary isoflavone excretion. The average urinary excretion of daidzein (46.4%) was nearly twofold that of genistein (28.7%), which agreed with the average fecal metabolism results. However, with relatively few volunteers participating in the prescreening, this study included a few subjects with relatively long or short fecal incubation half-lives (e.g., D1⁄2 = 21.2 h and G1⁄2 = 16.3 h for one subject; D1⁄2 = 7.1 h and G1⁄2 = 4.1 h for another subject, Table 1). Individual urinary excretions of isoflavones varied widely (4.5–8-fold).

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**TABLE 3**

**Plasma concentration of isoflavones in women and men after a single dose of 4.5 \( \mu \)mol total isoflavones/kg body weight from soymilk or soygerm**

<table>
<thead>
<tr>
<th>Time post-dose, h</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Soymilk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein2</td>
<td>ND³</td>
<td>1.04 ± 0.61b</td>
</tr>
<tr>
<td>Genistein</td>
<td>ND</td>
<td>1.70 ± 1.01a</td>
</tr>
<tr>
<td>Glycitein</td>
<td>ND</td>
<td>0.20 ± 0.08c</td>
</tr>
<tr>
<td>Soygerm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>ND</td>
<td>1.63 ± 1.03x</td>
</tr>
<tr>
<td>Genistein</td>
<td>ND</td>
<td>0.51 ± 0.19y</td>
</tr>
<tr>
<td>Glycitein</td>
<td>ND</td>
<td>0.73 ± 0.22y</td>
</tr>
</tbody>
</table>

1 Values are means ± sd, n = 6 for female soymilk feeding and n = 7 for male soymilk and both male and female soygerm feeding.
2 Plasma samples from soymilk and soygerm were statistically analyzed separately. Values in a column for either soymilk or soygerm with different superscripts are significantly different (P< 0.05).
3 ND = not detectable at a detection limit of 0.05 µmol/L.

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**TABLE 4**

**Human urinary isoflavone excretion as a percentage of dose ingested after a single dose of 4.5 \( \mu \)mol total isoflavones/kg body weight from soymilk versus soygerm**

<table>
<thead>
<tr>
<th>Time interval after dosing, h</th>
<th>% ingested dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td></td>
</tr>
<tr>
<td>6-12</td>
<td></td>
</tr>
<tr>
<td>12-24</td>
<td></td>
</tr>
<tr>
<td>24-48</td>
<td></td>
</tr>
<tr>
<td>Total, 0-48</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± sd. For soygerm intake results, n = 14; for soymilk intake results, n = 13.
2 The amount of each isoflavone compound intake was considered as 100%.
3 Values in a column with different superscripts are significantly different (P< 0.05).
URINARY DISPOSITION OF ISOFLAVONES

fold) even with selection of subjects by fecal degradation phenotype. Dietary patterns may be able to alter gut motility and fecal isoflavone degradation. For example, increasing insoluble dietary fibers would increase fecal bulk and decrease gut transit time (Jenkins et al. 1986) and microorganism populations, which would influence isoflavone metabolism and absorption. In a diet-controlled study, Karr et al. (1997) fed different levels of soy protein that contained 2.52 μmol daidzein/g protein and 4.07 μmol genistein/g protein to 14 subjects in four 9-d diet treatment periods. Urinary excretion of genistein varied by as much as 12-fold and daidzein by as much as 15-fold within diet treatments. In our study, except for a controlled amount of soy foods, diet intake was ad libitum. Compared with Karr’s study, the individual isoflavone urinary excretion varied within a lesser range. Overall, the subject screening method we used seemed to minimize the individual isoflavone excretion variability to some extent. Screening methods similar to ours may be important in understanding the role of gut microflora in phytochemical metabolism and for validation of the health effects of soy isoflavones and related phytochemicals. Further characterization of this screening technique is needed.

Glycitein has an -OCH₃ group at the 6-position, and neither glycitein nor daidzein have a 5-OH. This structural difference from genistein may result in less microfloral degradation than for genistein. Griffiths and Smith (1972) reported that isoflavones and flavonoids that possess a hydroxyl group in the 5-position of the A-ring, such as genistein, are much more susceptible to C-ring cleavage by rat gut bacteria. The more isoflavones are broken down by bacteria, the less isoflavones would be detected in urine. This may explain why less genistein was recovered from urine and could also explain why genistein had a shorter half-life than daidzein when anaerobically incubated with fecal samples in vitro (Xu et al. 1995). Gut microfloral isoflavone metabolites, such as equol (Adlercreutz et al. 1982; Axelsson et al. 1982), and O-desmethylangolensin (Adlercreutz et al. 1981; Bannwart et al. 1984), metabolites of daidzein; and p-ethylphenol (Griffiths and Smith, 1972), a metabolite of genistein, were identified. The metabolites of glycitein are unknown. It will be of great interest to develop methods to measure glycitein metabolites and to study the potential biological activity of both glycitein and its metabolites.

When the molar ratio of isoflavones fed was 1.1 genistein:0.2 glycitein (from soymilk, Table 1), plasma concentrations of genistein were significantly higher than that of daidzein, and plasma glycitein was very low. These patterns were similar to the soymilk feeding study of Xu et al. (1994). When soygerm (isoflavone molar ratios of 0.25 genistein: 1 daidzein: 0.8 glycitein, Table 1) was fed, plasma glycitein concentrations were significantly lower than daidzein concentrations in women, but plasma glycitein and genistein did not differ. In men, only at 6 h after feeding soygerm did plasma isoflavones correspond with soygerm isoflavone concentrations (daidzein > glycitein > genistein). The plasma concentrations at the 2 time points partially reflected dietary contents of isoflavones as well as individual metabolic differences. For example, subjects 10 and 11 had the highest fecal degradation half-life for daidzein (Table 2), and their plasma concentrations and total urinary excretions of daidzein after soygerm ingestion were also the largest, with 3.10 μmol/L, 60.3% (subject 10) and 3.06 μmol/L, 65.1% (subject 11), respectively. However, the reasons for the observed gender and time differences (Table 3) are unclear. Plasma pharmacokinetic stud-

ies after an oral dose of nearly equal amounts of genistein and daidzein fed to male subjects (Watanabe et al. 1998) showed that more genistein appeared in the circulation than did daidzein. Our soymilk feeding study, which had proportions of daidzein:genistein similar to that used by Watanabe et al. (1998), yielded similar results for both plasma and urinary excretion (Tables 3 and 4). After soygerm feeding, plasma genistein and glycitein concentrations at 6 h (in women) and 24 h (in both genders) did not differ, although the intake of glycitein was about four times that of genistein. If glycitein pharmacokinetics followed the same pattern as genistein and daidzein (King and Bursill 1998, Watanabe et al. 1998), lower plasma concentrations of glycitein than daidzein would be expected because a greater proportion of glycitein dose than that of daidzein was found in urine. According to retention times of the isoflavones on our reverse phase HPLC system, daidzein is more water-soluble than glycitein, and glycitein is more water-soluble than genistein. According to the percentage of ingested genistein or daidzein in urine did not differ between the isoflavone sources.

The present study showed that the urinary disposition of daidzein was different, with more glycitein excreted than daidzein and more daidzein excreted than genistein. A detectable amount of glycitein appeared in plasma even after soymilk feeding. Although glycitein is a minor isoflavone, these results suggest that determining biological effects of glycitein would be worthwhile.

LITERATURE CITED


