Nutrient Metabolism

Dietary Fructooligosaccharides Modify Intestinal Bioavailability of a Single Dose of Genistein and Daidzein and Affect Their Urinary Excretion and Kinetics in Blood of Rats

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ABSTRACT The influence of dietary fructooligosaccharides (FOS) on bioavailability of genistein and daidzein in rats was estimated by measuring their concentrations in plasma collected from three different veins and in urine after a single intragastric administration of isoflavone conjugates. Sprague-Dawley male rats (6 wk old, n = 22) were fed a purified control (AIN-93G) diet or a FOS diet (AIN-93G + 5% FOS) for 7 d. A single dose of soy isoflavone conjugates, i.e., 8.5 mg as genistein and 33 mg as daidzein/kg body, was administered via a stomach tube at d 5. Blood samples were collected after administration via catheters in the portal and central veins and by puncture of the tail vein. The isoflavones in plasma and urine were analyzed by time-resolved fluoroimmunoassay. The genistein concentration in the portal blood increased rapidly, reaching a peak of 3.5 μmol/L in both groups at 1 h after administration. The concentrations in the central and tail venous blood were approximately half of those in the portal blood. In the FOS-fed group, both genistein and daidzein remained detectable at 24 and 48 h in the tail venous plasma. The urinary excretion of both isoflavones in the 24- to 48-h period after administration was significantly higher in the FOS-fed group than in the control group. The difference between the portal and central veins indicated hepatic uptake, probably leading to conjugation of aglycones and excretion into bile. FOS modified the absorption and enterohepatic recirculation of isoflavones. J. Nutr. 131: 787–795, 2001.

KEY WORDS: • fructooligosaccharides • genistein • daidzein • rats • time-resolved fluoroimmunoassay

Several constituents of plants have estrogen-like physiologic effects. These constituents are called phytoestrogens (Munkies et al. 1998, Price and Fenwick 1985). Isoflavones (genistein and daidzein) in soybeans and lignans (enterolactone and enterodiol) formed in the intestine from their precursors in various grains, seeds, fruits, some vegetables and tea are commonly known phytoestrogens (Adlercreutz and Mazur 1997, Mazur et al. 1998). Dietary phytoestrogens may prevent sex hormone–related diseases, such as breast cancer and prostate cancer, through mechanisms that are insufficiently documented at present (Adlercreutz et al. 1982, Adlercreutz 1984 and 1990, Adlercreutz and Mazur 1997, Attalla et al. 1997, Landstrom et al. 1998). On the other hand, they may also prevent postmenopausal osteoporosis through an estrogenic effect (Arjmandi et al. 1996). For instance, intake of soy products (Arjmandi et al. 1998, Omi et al. 1994), genistein (Anderson et al. 1998, Ishimi et al. 1999), daidzein or other phytoestrogens (Draper et al. 1997, Ishida et al. 1998) has been found to prevent postovariectomized bone loss in rats and mice. It is important to clarify the levels of phytoestrogens in the blood attained through daily intake. Japanese people consume soy products containing ~30–50 mg of isoflavonoids daily, and this appears to play an important role in the prevention of cancer (Arai et al. 2000, Kimira et al. 1998, Watanabe et al. 1997). However, few studies regarding the kinetics of isoflavones in blood have been reported. Moreover, the intestinal environment is likely to influence their absorption. Almost all phytoestrogens in food exist as glycosides, such as genistin and daidzin; thus, to obtain physiologic activity, it is necessary to hydrolyze the glycosidic bonds for intestinal absorption to occur (Adlercreutz and Mazur 1997, Murkies et al. 1998, Price and Fenwick 1985). These glycosidic bonds are hydrolyzed by glucosidases of intestinal bacteria, such as Lactobacilli, Bacteroides and Bifidobacteria (Hawksworth et al. 1971, Xu et al. 1995). Fructooligosaccharides (FOS), a mixture of indigestible and fermentable sugars, have beneficial effects. FOS stimulate the growth of bifidobacteria in the intestine (Hidaka et al. 1986), increase Ca, Mg and Fe absorption and enhance bone calcium stores in rats (Ohta et

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al. 1995a, 1996 and 1998). Many of the effects of FOS feeding take place in the large intestine (Ohira et al. 1994, 1995b and 1997). Consequently, we postulate that dietary FOS may affect bioavailability of phytoestrogen glycosides and therefore improve their absorption form in the gut.

In this study, the kinetics of isoflavones in rats fed a FOS-supplemented diet or a control diet were examined by measuring genistein and daidzein concentrations in blood collected from the portal, central and tail veins, and by measuring urinary excretion over a 48-h period after a single administration of soy isoflavones.

**MATERIALS AND METHODS**

**Experimental procedure.** Male Sprague-Dawley rats (6 wk old; n = 22, Clea Japan, Tokyo, Japan) were housed in individual stainless steel wire-mesh cages in a room at 25°C and 55% relative humidity with a 12-h light:dark cycle; rats had free access to food and water. This study was approved by the Animal Studies Committee of Meiji Seika Bioscience Laboratories, and rats were maintained in accordance with the guidelines for the care and use of laboratory animals of Meiji Seika Bioscience Laboratories.

The rats were fed a pelleted diet (MF, oriental Yeast, Tokyo, Japan) for a 1-wk adaptation period. The rats (n = 22) were randomly assigned to two groups of 11 rats each, a purified control diet (AIN-93G) group and the FOS diet (AIN-93G + 5% FOS) group (Reeves et al. 1993). The composition of each of the diets is shown in Table 1. The experimental period was 7 d. All rats were fed 15 g diet/d on d 1 and 2 and 20 g/d on d 3–7, and were allowed free access to distilled water throughout the experimental period. Five rats from each group were used for sampling portal and central venous blood. The remaining 6 rats in each group were used for sampling tail venous blood.

On d 3 of experimental feeding, two catheters were inserted in rats under sodium pentobarbital anesthesia to sample portal and central venous blood. The tip of a portal cannula (silicone tube 0.5-mm i.d., with a 20-mm polyethylene tip SP-31, Natume, Tokyo, Japan) was inserted directly into the portal vein. A central venous cannula was inserted into the jugular vein and located in the central vein 3 cm from the insertion. The cannula was filled with heparinized (30 g/L) saline solution except at sampling times. These tubes were fastened to the abdominal wall, and the tip of the tube placed behind the neck under the skin without a harness to avoid any stress.

Isoflavone conjugates were administered to all rats in this experiment. Fujiflavone P40 [isoflavone content: 40% (daidzin, malonyl-daidzin, acetylglucosyl-daidzin and daidzein account for 20.4, 0.1, 1.1 and 0.3%, respectively; genistein, acetylglucosylgenistein and genistein account for 4.6, 0.3 and 0.1%, respectively; and glycitin and glycitein together account for ~1%)] was obtained from Fujikobo, Kobe, Japan. FOS (Meioligo-P, Meiji Seika Kaisha, Tokyo, Japan) is a mixture of 42% 1-kestose, 46% nystose and 9% 1F-β-fructofuranosylnystose.

Isoflavone (Fujiflavone P40) for administration to all rats was freshly prepared as a 1 mL suspension in water and a single dose [isoflavones: 100 mg (conjugates)/kg body (8.5 mg as genistein and 33 mg as daidzein)] was administered via a stomach tube. The rats were not restricted from eating throughout the experiment.

Two days after catheter implantation, blood was collected through the silicone tube connected on the neck to the portal and central veins. Blood (100 μL) was collected at 0, 1, 3, 6, 24 and 48 h after administration of isoflavones from the portal and central veins of 5 rats in each diet group under unanesthetized and unrestrained conditions; for the other rats (n = 6), blood was collected from the tail vein at these same times by the method of Hara et al. (1984).

Urine samples were collected during the periods 0–24 h and 24–48 h postadministration. NaN3 (0.003 mol/L) and ascorbic acid (~1 g/L) were added to the urine storage bottles to prevent oxidation of isoflavones. Each urine sample was stored separately in a bottle at ~80°C until the assay.

**Reagents.** Bovine serum albumin (BSA) and sulfates (EC 3.1.6.1; catalog no. S-9626) were purchased from Sigma Chemical (St Louis, MO). β-Glucuronidase (EC 3.2.1.31; catalog no. 1585665) was obtained from Boehringer (Mannheim, Germany). The assay buffer was 50 mmol/L Tris·HCl buffer, pH 7.8, containing (per L) 8.78 g NaCl, 0.5 g sodium azide, 5 g BSA and 0.1 g Tween 40. For enzymatic hydrolysis of the isoflavone conjugates in urine, 0.1 mol/L acetate buffer, pH 5.0, was used. Standards of genistein and daidzein were synthesized as previously described (Adlercreutz et al. 1986, Wahala and Hase 1991).

**Time-resolved fluorimunnoassay (TR-FIA) for measuring serum and urinary genistein and daidzein.** One milliliter of 0.1 mol/L acetate buffer (pH 5.0) containing 200 U/L β-glucuronidase and 2000 U/L sulfatase (hydrolysis reagent) was added to plastic tubes containing 50 μL of urine. The samples were mixed and incubated overnight at 37°C; 20 μL of the resulting solution was used for TR-FIA. (Adlercreutz et al. 1998 and 1999, Uehara et al. 2000, Wang et al. 2000).

Immunogen synthesis, immunization and labeling of isoflavonoid derivatives with europium chelate in the case of genistein and daidzein were described previously (Uehara et al. 2000, Wang et al. 2000).

The TR-FIA methods used for assay of isoflavones in plasma and urine are shown in Figure 1. Before the assay, microstrips coated with goat anti-rabbit immunoglobulin G were prewashed using 1296–026 DELFIA platewash (Wallac, Oy Turku, Finland). Of the standard serum or hydrolyzed urine samples, 20 μL was pipetted into the microstrips; then 100 μL of antiserum in 50 mmol/L Tris·HCl buffer containing 5 g/L BSA (pH 7.8) for genistein or daidzein, (antiserum diluted 1:50,000 and 1:40,000, respectively) and 100 μL of europium-labeled genistein or daidzein (diluted 1:40,000 and 1:40,000, respectively) were added per well. The strips were placed on a 1296–038 DELFIA shaker (Wallac) and shaken slowly at room temperature for 90 min, then washed with a DELFIA platewasher using the no. 29-T3 program. DELFIA enhancement solution (200 μL) 1244–105 (Wallac) was added to each well and the strips were shaken slowly for an additional 5 min. Fluorescence was read using the DELFIA Victor 1420 multilabel counter and the accompanying software, version 1.0, for data analysis. The final result was calculated by means of the following formula:

Final result = Concentration (read) × dilution factor (nmol/L)

The TR-FIA method, including hydrolysis and extraction, gives the most specific results for plasma or serum, but we used a modified method that omitted extraction of the unhydrolyzed plasma because the quantities of several blood samples were insufficient for extraction. The direct method for assay of genistein and daidzein in serum or plasma measures only the free aglycones, the 4′-monosulfates and the 4′-monoglucuronides, and gives lower (~30%) in rats) values than those obtained by gas chromatography–mass spectrometry (GC-MS) analysis.

Urinary isoflavones were analyzed by a method including a hy-

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**TABLE 1**

Composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>5% FOS1 diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg diet</td>
<td>g/kg diet</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>532</td>
<td>532</td>
</tr>
<tr>
<td>Corn oil</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mixture2</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>FOS1</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

1 Fructooligosaccharides (FOS, Meioligo-P, Meiji Seika Kaisha, Tokyo, Japan; concentration of oligosaccharides was >95% of total mixture).
2 Prepared according to AIN-93 formulation (Reeves et al. 1993).
where \( t \) is of isoflavones were calculated by the following formula:

\[
\text{Isoflavone concentration at } t = (\text{TR-FIA values by means of the formula:})
\]

The mean value of urinary (hydrolyzed) genistein was much higher by the TR-FIA method than by the GC-MS method, but that of daidzein was similar by both methods. However, there was a significant difference (P < 0.05), each value was converted to the logarithmic value. Three-way ANOVA was performed to determine the main effects of diet, the time course of changes, and the interactions with respect to isoflavone concentrations in the portal and central venous blood. Two-way ANOVA was used to determine the main effects of diet and the time course of changes, and the interactions with respect to isoflavone concentrations in blood from the tail vein and hepatic uptake. Tukey’s test (Dawson-Saunder and Trapp 1994) was used for comparison of means within a factor. The effect of absorption of isoflavones calculated from the areas under the plasma concentration time course curves in the case of blood from the portal, central and tail veins, and the effect of FOS feeding on food intake, body weight gain and cecal wet weight, \( pH \) and concentrations of organic acids were examined by Student’s unpaired \( t \) test; the equality of variance was determined using Levene’s test. Differences were considered significant at \( P < 0.05 \). All statistical analyses were performed using the SPSS package program version 6.1 J (Chicago, IL) and Excel 97 with Microsoft Windows 95 or 98.

RESULTS

Food intake and animal weight. There were no significant differences in food intake (130 g for all rats in 7 d) and body weight gain between the control (50.2 ± 1.5 g) and FOS-fed (49.3 ± 4.6 g) groups.

Cecal wet weight, \( pH \) and concentrations of organic acids. After 1 wk of feeding, cecal contents wet weight was significantly greater in rats fed the FOS diet than in those fed the control diet (\( P < 0.01 \)). Cecal \( pH \) was significantly lower in rats fed FOS (\( P < 0.01 \)) compared with the control. Lactate, propionate and butyrate concentrations were significantly higher in rats fed the FOS diet than in those fed the control diet (\( P < 0.05 \) or 0.01). There was no significant difference between the FOS-fed and control rats in concentrations of succinate and acetate (Table 2).

Portal and central venous plasma genistein and daidzein concentrations. Genistein concentrations in portal and central venous plasma after administration of a single dose of isoflavones are shown in Figure 2 A and B, respectively. Three-way ANOVA showed that the three main effects (diet, time and vein) and the interaction (time and vein) were significant (\( P < 0.002 \)) for genistein (Table 3). In the control rats, the genistein concentrations in portal blood reached a peak at 1 h after administration (3.5 ± 0.7 \( \mu \)mol/L) and decreased linearly thereafter. In the FOS-fed group also, the

**TABLE 2**

Cecal contents wet weight, \( pH \) and concentrations of organic acids in rats fed the control diet or the 5% fructooligosaccharide (FOS) diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cecal wet contents</th>
<th>pH</th>
<th>Succinate</th>
<th>Lactate</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>(mol/g wet cecal contents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.90 ± 0.10</td>
<td>6.75 ± 0.07</td>
<td>1.40 ± 1.13</td>
<td>4.68 ± 0.86</td>
<td>23.9 ± 3.5</td>
<td>3.41 ± 0.79</td>
<td>1.92 ± 0.41</td>
</tr>
<tr>
<td>FOS</td>
<td>4.61 ± 0.27*</td>
<td>5.21 ± 0.12*</td>
<td>0.64 ± 0.45</td>
<td>31.0 ± 10.0*</td>
<td>18.2 ± 2.2</td>
<td>7.81 ± 1.32*</td>
<td>13.1 ± 2.4*</td>
</tr>
</tbody>
</table>

* Significantly different from the control rats by Student’s \( t \) test after Levene’s test for equality (\( P < 0.05 \)).

1 Values are means ± SEM, \( n = 8–9 \).
Two-way ANOVA was used to test the main effects of interaction of feeding diets and the time course of changes in plasma isoflavones, two-way ANOVA was used. NS: Not significant, $P > 0.05$.

### Table 3

Three- and two-way ANOVA of genistein and daidzein concentrations in portal, central and tail venous plasma, and hepatic uptake in rats fed the control diet or the 5% fructooligosaccharide (FOS) diet.

<table>
<thead>
<tr>
<th></th>
<th>Diet (D)</th>
<th>Time (T)</th>
<th>Vein (V)</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D x T</td>
<td>D x V</td>
<td>T x V</td>
<td>D x T x V</td>
</tr>
<tr>
<td><strong>Three-way ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal and central veins</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.002$</td>
<td>NS</td>
</tr>
<tr>
<td>Genistein</td>
<td>NS</td>
<td>$P = 0.0001$</td>
<td>$P = 0.009$</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td>Daidzein</td>
<td>NS</td>
<td>$P = 0.0001$</td>
<td>$P = 0.009$</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td><strong>Two-way ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>$P = 0.004$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td>Daidzein</td>
<td>NS</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td>Hepatic uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>NS</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
</tr>
<tr>
<td>Daidzein</td>
<td>NS</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
<td>$P = 0.0001$</td>
</tr>
</tbody>
</table>

1 To test the main effects of interaction of feeding diets, the time course of changes in plasma isoflavones and the type of veins from which blood was collected, three-way ANOVA was used.

2 To test the main effects of interaction of feeding diets and the time course of changes in plasma isoflavones, two-way ANOVA was used. NS: Not significant, $P > 0.05$.
the genistein concentration was elevated at 1 h and did not change until after 6 h of administration. The genistein concentrations were significantly higher in the FOS-fed group than in the control group at 24 and 48 h after administration (P < 0.05). The daidzein concentration reached a maximum at 1 h in the control group (2.2 ± 0.6 μmol/L) but the peak area was delayed until 3–6 h postadministration in the FOS-fed group.

The central and the tail venous blood concentrations were correlated for both genistein (r² = 0.887, P < 0.001) and daidzein (r² = 0.829, P < 0.001).

**Absorption of genistein and daidzein.** Genistein absorption calculated from the areas under the plasma concentration curves for portal, central and tail venous blood in rats fed the control diet and the FOS diet during the periods 0–6 h, 6–48 h and 0–48 h after isoflavone administration is shown in **Table 4**. On the basis of the concentrations in plasma from the three veins of the FOS-fed group compared with the control group, no significant differences in the absorption of genistein existed during the 0- to 6-h period after administration. During the 6- to 48-h period after administration, the genistein areas under the concentration curves of the central and tail venous plasma concentrations were significantly higher in the FOS-fed group than in the control group (P < 0.05). During the 0- to 48-h period, the genistein areas under the concentration curves in tail venous plasma were also higher in the FOS group.

**TABLE 4**
The absorption of genistein and daidzein calculated from areas under the plasma concentration curves at specific time periods in portal, central and tail venous blood in rats fed the control diet or the 5% fructooligosaccharide (FOS) diet.

<table>
<thead>
<tr>
<th>Veins</th>
<th>Diet</th>
<th>0–6 h μmol/L</th>
<th>6–48 h μmol/L</th>
<th>0–48 h μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal</td>
<td>Control</td>
<td>13.2 ± 2.8</td>
<td>10.6 ± 1.7</td>
<td>23.8 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>FOS</td>
<td>12.4 ± 2.1</td>
<td>18.6 ± 3.7</td>
<td>31.0 ± 7.8</td>
</tr>
<tr>
<td>Central</td>
<td>Control</td>
<td>6.36 ± 1.08</td>
<td>5.72 ± 0.91</td>
<td>12.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>FOS</td>
<td>6.80 ± 0.91</td>
<td>12.5 ± 2.7*</td>
<td>19.3 ± 2.9</td>
</tr>
<tr>
<td>Tail</td>
<td>Control</td>
<td>6.39 ± 0.39</td>
<td>5.68 ± 0.94</td>
<td>12.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>FOS</td>
<td>6.15 ± 0.71</td>
<td>13.2 ± 1.7**</td>
<td>19.3 ± 2.0**</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5. *Significantly different from the control rats by Student’s t test after Levene’s test for equality (P < 0.05).
FOS-fed group than in the control group (P < 0.01). For daidzein, there was a significant difference between areas under the concentration curves of the FOS-fed and control groups during the 0–48-h period in the tail vein samples (P < 0.05) (Table 4).

Hepatic uptake of genistein and daidzein. We assessed the hepatic uptake of isoflavones into the liver by comparing the concentrations in portal and central venous plasma (Fig. 5 A and B). Two-way ANOVA showed that time was a significant effect (P < 0.0001) for genistein and daidzein (Table 3). The hepatic uptake of both genistein and daidzein was greater 1 h after administration of a single dose of isoflavones compared with the values at other time points. There were no significant differences between the control and the FOS-fed groups at any time.

Urinary excretion of genistein was not different during the 0- to 24-h period between the two dietary groups after administration of a single dose of isoflavones. However, the excretion was significantly higher in the FOS-fed group (Urine-2 in Table 5) than that in the control group during the 24- to 48-h period after administration (P < 0.01). The excretion of daidzein did not differ between rats fed the control and FOS diets at any time. The amount of genistein recovered in the urine was ~11% and 14% in the control and FOS-fed groups, respectively. The amount of daidzein recovered in urine was 21–23% in both groups.

**DISCUSSION**

We examined the changes in circulating levels of isoflavones after intragastric administration of a single dose of isoflavones to rats by measuring the genistein and daidzein concentrations in portal, central and tail venous plasma, and the urinary excretion of these compounds. Our assumptions were as follows: 1) the concentrations of genistein and daidzein in portal blood should be a reflection of the degree of absorption; 2) the concentrations in the tail venous blood represent the peripheral blood concentrations; 3) the difference between the portal and central venous blood concentrations is indicative of hepatic uptake and biliary excretion; and 4) the difference between the central and tail venous blood concentration reflects the elimination of isoflavone by the kidneys.

Phytoestrogens occur mainly as glucuronide or sulfate conjugates in plasma and urine. We measured only some of the isoflavone conjugates and free aglycones present in plasma by TR-FIA, and this assay was carried out directly without hydrolysis and extraction. The conjugates are mainly 4′-monosulfate and monoglucuronides, but some other conjugates seem to have been included because the main isoflavone conjugates in rat plasma and urine are 7-O-glucuronides (Sfakianos et al. 1997). We compared the values in direct and extracted samples of rat plasma (n = 15), and the mean values by the direct method were 70% of the values by the extracted method (data not shown). Therefore, the direct values are underestimated, but the difference in isoflavone concentrations between the different venous plasmas and between rats fed the FOS and control diets could be determined. Our results suggest, however, that the 4′-conjugates of genistein and daidzein occur in relatively large amounts after an isoflavone load.

Isoflavone conjugates may be deglycosylated by the strong acid in the stomach. Enzymes such as β-glucosidase produced by the intestinal microflora before absorption are considered to

**TABLE 5**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Genistein</th>
<th>Daidzein</th>
<th>Genistein</th>
<th>Daidzein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–24 h</td>
<td>24–48 h</td>
<td>0–48 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>μmol</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine-1</td>
<td>Control</td>
<td>0.70 ± 0.07</td>
<td>0.12 ± 0.02</td>
<td>0.83 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>FOS</td>
<td>0.82 ± 0.07</td>
<td>0.16 ± 0.03</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>Urine-2</td>
<td>Control</td>
<td>0.78 ± 0.10</td>
<td>0.11 ± 0.01</td>
<td>0.89 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>FOS</td>
<td>0.86 ± 0.10</td>
<td>0.22 ± 0.02*</td>
<td>1.08 ± 0.11</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5–6. * Significantly different from the control rats by Student’s t test after Levene’s test for equality (P < 0.05).
2 Urine-1: Urine samples from the rats taking portal and central venous blood; Urine-2: Urine samples from the rats taking tail venous blood.
be responsible for the hydrolysis. Recently it was shown in
humans that genistein and daidzein can be detected in blood
within 15 min after consumption of soy protein (textured
vegetable protein) (Bowey et al. 1998), suggesting hydrolysis
in the upper gastrointestinal tract. Day et al. (1998) reported
that genistein 7-glucoside and daidzein 7-glucoside are rapidly
deglycosylated by cell-free extracts from human small intestine
and liver, and they suggested that deglycosylation of isofla-
vones by human cytosolic β-glucosidase could be an important
first step in the metabolism of these compounds, independent
of deglycosylation by the colonic microra. Ioku et al. (1998)
also measured β-glucosidase activity in the small intestine of
rats using flavonoid glucosides as substrates, and their findings
suggested that dietary flavonoid glucosides are hydrolyzed pri-
marily in the jejunum, thereby liberating aglycones. In this
study, the concentrations of both genistein and daidzein in
portal blood rapidly reached a peak (within 1 and 3 h, respec-
tively) after a single dose of isoflavones. This finding indicates
that these isoflavones are readily absorbed in the small intest-
tine after intragastric administration. Piskula (2000) reported
that in tail vein plasma, total daidzein and genistein reached
maximum concentrations in food-deprived (intragastric ad-
mistration) rats at 7 min after administration of their agly-
cones.

Twenty-four hours after administration, the concentrations
of genistein and daidzein in blood from all three veins were
< 10% of the peak levels observed. The intestinal transit time
of the diet in rats has been estimated to be ~20–30 h (Sak-
aguchi et al. 1987). The length of time until disappearance of
both isoflavones was similar to the intestinal transit time.
These results suggest that in rats, the peripheral blood con-
centration reflects mainly intestinal absorption, and that
genistein and daidzein do not remain in the blood and prob-
ably do not remain in the body for a long time.

In the case of both genistein and daidzein, good correlations
between the portal and central venous plasma concentrations
were observed in this study. Indeed, the central venous plasma
isoflavone concentrations were nearly half the portal venous
concentration during the 1- to 12-h period after administra-
tion. This result indicates that about half of the genistein
and/or daidzein absorbed was taken up into the liver, and this
metabolic pathway is similar to that of endogenous estrogens
(Adlercreutz and Martin 1980). Also, good correlations be-
tween the central and tail venous blood concentrations were
observed for both genistein and daidzein. According to the
results of this study, 15% of plasma genistein and 35% of
plasma daidzein disappeared from the peripheral circulation by
urinary excretion.

Tew et al. (1996) suggested that genistein has lower bio-
availability than daidzein because of the low urinary output of
genistein. However, Sfakianos et al. (1997) examined the
intestinal absorption, biliary excretion and metabolism of
genistein using adult female rats fitted with indwelling biliary
cannulas and found that genistein was absorbed from the intestines very well; it was excreted mostly into the bile, and
only a small portion appeared in the urine. Both genistein and
daidzein are converted to sulfate and glucuronide conjugates in
rats. Daidzein may be eliminated more rapidly in the urine
than genistein. Estrogens with three hydroxyl groups are more
abundantly excreted into the bile (Adlercreutz and Luuk-
kainen 1967); because of the three hydroxyl groups, genistein
is more likely than daidzein to be excreted rapidly in bile. In
other words, it seems that the isoflavonoid conjugates in
peripheral blood is probably regulated through biliary excre-
tion. A likely reason for the greater excretion of compounds
with three hydroxyl groups compared with those with two is
the formation of polar double conjugates (Adlercreutz et al.
1973).

The recovery of genistein in urine was 10–12% and 13–15% of the amount administered in the case of the control and
FOS-fed groups, respectively. The recovery of daidzein in urine
was ~23% in both groups. Landstrom et al. (1998) reported
the urinary recovery during two metabolic periods was 1 and
3% for genistein and 11 and 28% for daidzein in rats after
tumor transplantation and soy intake. In the study of King
(1998), the recovery of genistein and daidzein in urine of rats
was 11.9 and 17.4%, respectively.

The data obtained in this study demonstrate for the first
time that FOS modify the bioavailability of isoflavones. Through observation of cecal contents weight, pH and com-
position of organic acids, we concluded that the rats fed the
FOS diets for 7 d were almost fully adapted to FOS. In this
study, genistein and daidzein remained detectable for a signif-
cantly longer period in the tail venous blood of rats fed the
FOS diet compared with the controls. The absorption of
genistein, as calculated on the basis of the concentrations in
portal, central and tail venous blood during the 6- to 48-h
period after administration, was significantly higher in rats fed
the FOS diet than in controls, but the absorption of daidzein
did not differ. This indicates that FOS enhanced the entero-
hepatic recirculation and/or large intestinal absorption of
genistein. Adlercreutz (1962) suggested that the proportions
of estrogen metabolites in the enterohepatic recirculation already
excreted into bile increased transiently in the 6- to 10-h period
after the start of oral administration of estradiol. In this study,
a significant difference between the control and FOS-fed
groups in the genistein concentration in the tail venous blood
came evident 6 h after administration of the isoflavones.
FOS are indigestible because they are resistant to hydrolysis by
mammalian enzymes, and they stimulate the growth of intes-
tinal bifidobacteria. In this manner, dietary FOS may increase
β-glucosidase activity in the large intestine (Hidaka et al.
1986). As indicated above, in FOS-fed rats, a portion of the
isoflavone-glucosides consumed was likely to have been deg-
lycosylated and absorbed as aglycones in the large intestine.
Our findings regarding urinary isoflavone excretion also sup-
port this hypothesis because the extent of excretion of both
genistein and daidzein was higher in rats fed the FOS diet than
in control rats during the 24- to 48-h period after administra-
tion. The relative absorption of genistein, calculated on the
basis of the urinary excretion, was ~20% higher in FOS-fed
rats than in control rats. FOS may be useful for maintaining
elevated blood levels of isoflavones, especially in the case of
genistein. Additionally, we speculate that FOS may enhance
the metabolism of isoflavones, particularly for daidzein to
metabolites such as equol and O-desmethylandolensin. This
could explain the difference observed between genistein and
daidzein concentrations in plasma when the two dietary groups
were compared. Isoflavonoids undergo extensive metabolism
by gut microflora (Heinonen et al. 1999). Considerable evi-
dence exists showing extensive interindividual variation in
isoflavone metabolism in humans (Lampe et al. 1998, Rowland
hydrate-rich diet increases equol production (Lampe et al.
1998, Rowland et al. 2000). However, rats are constitutive
equol producers in contrast to humans (King 1998, Landstrom
et al. 1998). FOS can change the composition of gut micro-
flora and may change the production of daidzein metabolites
in rats. However, further studies should be conducted.

In conclusion, with a single administration of isoflavones,
The skilful technical assistance of Adile Sameldin is acknowledged. We thank Tarja Nurmi for analysis of free aglycones of isoflavones in Fujifavone P40 after hydrolysis by HPLC to calculate the recovery of genistein or daidzein in urine, and Witold Mazur for valuable suggestions for this paper.

LITERATURE CITED


