High Fiber Diet Supplemented with Rice Bran Hemicellulose May Reduce Daidzein Absorption in Mice

Motoi TAMURA1*, Takashi IWAMI1, Kazuhiro HIRAYAMA2 and Kikuji ITOH2

1 National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan
2 Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

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Much attention has focused on the health benefits of isoflavone. Investigating the absorption and metabolism of isoflavonoids is essential for understanding their biological activity. We explored the effect of a high fiber diet containing rice bran hemicellulose on the plasma and cecal isoflavonoids in mice. Mice were fed a 5% rice bran hemicellulose and 5% cellulose-0.1% daidzein diet (RBI diet) or a 5% cellulose-0.1% daidzein diet (CI diet) for 30 days. After the 30-day feeding period, the mice were sacrificed and blood and cecal contents were collected. The plasma isoflavonoids and plasma total cholesterol concentrations were measured. The plasma daidzein concentrations were significantly lower in the RBI group compared to the CI group. In contrast, there were no significant differences in the plasma equol concentrations or plasma total cholesterol concentrations between the RBI and CI groups. Binding affinity against isoflavonoids and/or the bulking effect in the gut might be related with the reduction in plasma daidzein concentration in the RBI group.

Keywords: rice bran hemicellulose, daidzein, equol

Introduction

Much attention has focused on the health benefits of soy-based foods, which have been largely attributed to isoflavone. Daidzin, glycitin, daizein (the aglycone of daidzin) and glycitein (the aglycone of glycitin) are isoflavone that are found in soy products. Human metabolism and excretion of isoflavone following the consumption of soy products exhibit considerable variation (Kelly et al., 1995; Xu et al., 1995). Equol is a bacterial metabolite of the widespread isoflavone daidzein (Bowey et al., 2003). These isoflavonoids are estrogenic compounds (phytoestrogens). In animals, phytoestrogens exert estrogenic effects on the central nervous system, induce estrus and stimulate the growth of the female genital tract (Lieberman, 1996). Investigating the absorption and metabolism of isoflavonoids is essential for understanding their biological activity. Some reports have found dietary fiber affects the bioavailability of isoflavones. One report suggests that dietary fiber or other components of a highfiber diet promote the growth and/or the activity of bacterial populations responsible for equol production in the colon (Lampe et al., 1998). Fiber intake and urinary excretion of lignans and equol correlated negatively with plasma percentage free estradiol (Adlercreutz et al., 1987). Recently, it has been reported that dietary konjac glucomannan may enhance the equol production in mice (Tamura et al., 2005). However, few reports have investigated the effects of the high concentration of dietary fiber on the bioavailability of isoflavone. As cholesterol excretion in the feces was influenced by rice hemicellulose in the diet, rice bran hemicellulose appears to be a functional food in the gut (Normand et al., 1984). In the present study, we explored the effect of a high fiber diet containing rice bran hemicellulose on the plasma isoflavonoids in mice.

Materials and Methods

Materials Rice bran hemicellulose was kindly provided by Boso Oil and Fat Co. (Tokyo, Japan). The daidzein and equol used as a standard for HPLC analysis was purchased from LC Laboratories (Woburn, MA, USA). β-Glucuronidase type H-5 was obtained from Sigma (St. Louis, MO, USA).

Treatment of animals Male Crj: CD-1 (ICR) mice (7...
weeks old) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). All mice were specific pathogen-free (SPF) and were housed in conventional conditions in our laboratory. The mice were randomly divided into two groups of seven animals each. The animals were housed in suspended stainless-steel cages with wire mesh bottoms in a room kept at 24 ± 0.5°C and a relative humidity of 65% with a 12-h light/dark cycle. They were fed an MF pelleted diet for two weeks. After two weeks, the diet was replaced with an AIN-93M diet for one week. Following the AIN-93M diet, mice were fed the rice bran hemicellulose-daidzein (RBI) diet or cellulose-daidzein (CI) diet for 30 days; all mice were pair-fed. The composition of each diet is shown in Table 1. The RBI diet contained 5% rice bran hemicellulose plus 5% cellulose and 0.1% daidzein (isoflavone). The CI diet contained 5% cellulose and 0.1% daidzein (isoflavone). Body weight and food consumption were measured during the experiment. After the feeding period, the unfasted mice were sacrificed at about 10:00 a.m. and blood and cecal contents were collected. All mice were alternately anesthetized. The blood samples were then centrifuged, and the plasma was stored at –80°C until HPLC analysis for isoflavonoids and cholesterol. This study was carried out in accordance with the Guidelines for Animal Care and Experimentation of the National Food Research Institute.

**Measurement of plasma cholesterol** The plasma total cholesterol concentrations were measured using a cholesterol C-test Wako kit (Wako Pure Chemical Industries) based on

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>AIN-93M diet (g/kg diet)</th>
<th>Rice bran-isoavone diet (g/kg diet)</th>
<th>Cellulose-isoavone (control) diet (g/kg diet)</th>
</tr>
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<tbody>
<tr>
<td>Cornstarch</td>
<td>465.692</td>
<td>414.692</td>
<td>464.692</td>
</tr>
<tr>
<td>Casein</td>
<td>140.0</td>
<td>140.0</td>
<td>140.0</td>
</tr>
<tr>
<td>α -Cornstarch</td>
<td>155.0</td>
<td>155.0</td>
<td>155.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>40.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Rice bran hemicellulose</td>
<td>—</td>
<td>50.0</td>
<td>—</td>
</tr>
<tr>
<td>Mineral mix (AIN-93M-MX)</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93-MX)</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Tert-butylhydroquinone</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Daidzein¹</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

¹Daidzein was purchased from LC laboratories (Woburn, MA, USA).
the cholesterol oxidase-phenol methods (Allain et al., 1974).

Analysis of plasma isoflavonoids The analysis of plasma isoflavonoids was performed as follows. A total of 200 µL plasma was added to 200 µL β-glucuronidase type H-5 solution (35 mg/mL, Sigma, MO, USA) in 0.2 M sodium acetate buffer (pH 5.0). Next, the mixture was incubated at 37°C in a shaking water bath for 2 h, followed by treatment with 3.60 mL methanol/acetic acid (100/5, v/v), vortexing for 30 s, sonication for 30 s, vortexing again for 30 s, and centrifugation at 5000 × g for 10 min at 4°C. The supernatants were transferred to round-bottom flasks and evaporated completely using a rotary evaporator. The sample was then dissolved within 400 µL of 80% methanol and filtrated through a 0.2-µm filter. For HPLC analysis, we injected 20 µL of each preparation into a 250 × 4.6 mm Capcell Pak C18 type MG 5-µm column (Shiseido, Tokyo, Japan). To detect isoflavonoids, a JASCO MD-1515 Photodiode array detector (JASCO, Tokyo, Japan) was used to monitor the spectral data from 200 to 400 nm for each peak. To measure the isoflavonoids, we used daidzein and equol as standard samples. We used the spectral data of 254 nm and 280 nm to quantify daidzein and equol, respectively. The mobile phase consisted of methanol/acetic acid/water (35:5:60, v/v/v). The running conditions of HPLC were as follows: column temperature, 40°C; flow-rate, 1 mL/min.

Statistics The data were expressed as the means ± SD. Data were analyzed using the SigmaStat for windows (Jandel Corporation, San Rafael, CA, USA) and t-test analysis.

Results

Body weight, food consumption, and cecal contents No significant differences in final body weight (g) were observed between the RBI group (44.7 ± 1.9) and the CI group (44.2 ± 3.4). No significant differences in food consumption (g/day) were observed between the RBI group (4.8 ± 0.1) and the CI group (4.8 ± 0.1). However, there were significant differences (p < 0.05) in the cecal contents between the RBI group and the CI group (Fig. 1).

Plasma total cholesterol concentration Plasma total cholesterol concentrations of two dietary groups are shown in Fig. 2. No significant differences in plasma total cholesterol concentrations were observed between the RBI and CI groups.

Plasma isoflavonoids Plasma concentrations of daidzein and equol in two dietary groups are shown in Fig. 3. The plasma daidzein concentrations were significantly lower in the RBI group compared to the CI group. In contrast, there were no significant differences in the plasma equol concentrations between the RBI and CI groups. Though the ratio of plasma equol/plasma daidzein tended to be high in the RBI diet group (16.9 ± 6.8) compared to the CI diet group (11.2 ± 6.9), no significant difference was observed in the ratio of plasma equol/plasma daidzein between the two dietary groups.

Discussion

The health benefits of soy-based foods that are receiving

![Fig. 1. Cecal contents of the mice in the RBI and CI groups. Values are means ± SD. *Significantly different (p<0.05) from the CI group.](image-url)
much attention have been largely attributed to the isoflavones daidzin and daizein (the aglycone of daidzin). Investigating the absorption and metabolism of isoflavonoids is essential for understanding their biological activity. In the present study, a high fiber daidzein diet containing rice bran hemicellulose resulted in a lower concentration of plasma daidzein in mice. Some dietary fiber affects serum estrogen, as has been reported after two months on a wheat bran-supplemented high-fiber diet; that is, premenopausal women exhibited significant reductions in serum estrone \((P < 0.002)\) and estradiol \((P < 0.02)\) (Rose et al., 1991).

In our experiment, plasma daidzein concentration was affected by rice bran hemicellulose diet supplementation. Thus, some dietary fiber may affect the bioavailability of estrogens as well as phytoestrogens, such as daidzein. In addition, the binding of estrogens to various fibers has been...
reported (Arts et al., 1991). This report revealed that linseed (91%), oats (83%), barley chaff (88%), and wheat bran (82%) were excellent binders of 17β-estradiol. Corn, rye, and white wheat flour had a lower binding capacity with a relatively low affinity. Moreover, an in vitro binding of estrone, 17β-estradiol, estradiol, dihydrotestosterone, and estrone-3-glucuronide by dietary fiber were measured. Wheat and oat bran were more likely to bind the estrogens than cellulose (Shultz and Howie, 1986). The binding affinity of estrogen and phytoestrogen seems to vary with different types of dietary fiber. It has been suggested that a diet high in wheat fiber decreases the bioavailability of genistein (Tew et al., 1996). In the report, the authors suggest that hydrophobic binding to the genistin of dietary fiber partially affects the bioavailability of genistein. The binding affinity of rice bran hemicellulose with daidzein may affect the bioavailability of daidzein. Equol is a bacterial metabolite of the widespread isoflavone daidzein (Bowey et al., 2003). It has been reported that human daidzein bioavailability depends upon the relative ability of gut microflora to degrade these compounds (Xu et al., 1995). The plasma ratio of plasma equol/plasma daidzein and plasma equol concentration seems to reflect the equol productivities of intestinal flora in the gut. Lampe et al. (1998) reported that dietary fiber or other components of a high-fiber diet may promote the growth and/or the activity of bacterial populations responsible for equol production in the colon. Some dietary fiber may affect equol production by intestinal flora. However, in our experiment conditions, rice bran supplementation to the diet did not affect plasma equol concentrations. Thus the binding affinity of rice bran hemicellulose to equol may counterbalance the effects of rice bran hemicellulose on equol production by the intestinal flora in the gut.

We used the isoflavone aglycone in our experiment. However, it has been reported that isoflavone absorption in aglycones differs from that in glycosides (Setchell et al., 2002). In addition, soy isoflavone aglycones are absorbed faster and in higher amounts than glucosides in humans (Izumi et al., 2000). These reports indicate that β-glucosidase activity in the gut is important for the bioavailability of isoflavone glycoside.

As wheat bran has been reported to increase β-glucosidase activity of the cecal contents of rat (Mallett et al., 1986), rice bran hemicellulose might also affect the bioavailability of isoflavone glycoside through the modification of β-glucosidase activity of the intestinal microflora. However, it has been reported that fecal bulking increases significantly in a dose-dependent manner with dietary fiber supplementation (Staniforth et al., 1991). In our experiment, the cecal content in the RBI diet group (consuming 10% dietary fiber) was significantly greater than that in the CI diet group (consuming 5% dietary fiber). Thus, bulking effects might have been greater in the RBI group than in the CI group, as the bulking effects in the gut by high dietary fiber contents might have partially resulted in a lower concentration of plasma daidzein in the RBI group. Although no significant differences between the plasma total cholesterol concentrations of the RBI group the CI group were observed, it has been reported that rice bran hemicellulose suppresses the elevation of serum cholesterol levels in rats fed a hypercholesterolemic diet containing 1% cholesterol (Aoe et al., 1989). As we measured the plasma endogenous cholesterol without cholesterol addition to the diet, rice bran hemicellulose may not have an endogenous cholesterol-lowering effect.

In conclusion, we first demonstrated that the ingestion of rice bran hemicellulose may reduce plasma daidzein concentration in mice. The binding affinity of rice bran hemicellulose against isoflavonoids and/or the bulking effect in the gut might have been related to the reduction of plasma daidzein concentration in the RBI group.

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References
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