Soy Isoflavone Aglycones Are Absorbed Faster and in Higher Amounts than Their Glucosides in Humans

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ABSTRACT Isoflavones are contained in soybean or soy foods in two chemical forms, i.e., aglycones and glucosides. We investigated the difference in the absorption of soy isoflavone aglycones and glucosides in humans. After a single, low dose intake (0.11 mmol), the highest isoflavone concentrations in plasma were reached 2 and 4 h after ingestion of aglycones and glucosides, respectively; subjects were four men (41 y old) and four women (45 y old). The highest plasma concentration after aglycone intake was more than two times greater than that after glucoside ingestion. In a similar manner, we then compared the plasma isoflavone concentration profiles after intake of a single, high dose of isoflavones (1.7 mmol) in eight subjects (four men, 40 y old; four women, 47 y old) and found the highest plasma concentration after aglycone intake was more than five times higher than that after glucoside intake. In both high and low dose intake tests, the plasma concentration of genistein was significantly higher than that of daidzein despite the similar levels of intake. After long-term (4 wk) intakes (0.30 mmol/d), we also measured the plasma concentration of isoflavones (eight men, 45 y old). After 2 and 4 wk, these concentrations remained >100% higher after ingestion of aglycones than of glucosides. The isoflavone aglycones were absorbed faster and in greater amounts than their glucosides in humans. Isoflavone aglycone-rich products may be more effective than glucoside-rich products in preventing chronic disease such as coronary heart disease. J. Nutr. 130: 1695–1699, 2000.

KEY WORDS: • *isoflavone absorption* • *aglycone* • *genistein* • *daidzein* • *humans*

Soybeans, which have long been part of the diet in Asian countries, contain a variety of biologically active compounds (Messina and Messina 1991). Interest in soy ingredients has increased recently all over the world. Epidemiologic studies have shown that the consumption of soybeans decreases the risk of various diseases and conditions, including breast cancer (Adlercreutz et al. 1991, Lee et al. 1991), prostate cancer (Severson et al. 1989), colon cancer (Watanabe and Koessel 1993), osteoporosis (Knight and Eden 1996), menopausal symptoms (Adlercreutz et al. 1992) and coronary heart disease (Clarkson et al. 1995). Isoflavones in soybean exist primarily as glucoside forms: 6″-*O*-malonylglucosides and 6″-*O*-acetylglucosides (Izumi et al. 1997, Kudou et al. 1991b, Naim et al. 1973, Ohta et al. 1979 and 1980, Walter 1941) and rarely as aglycone forms. Isoflavone aglycones (IFA) are contained in miso, natto and soy sauce, Japanese traditional fermented soy foods (Wang and Murphy 1994). The representative isoflavone glucosides (IFG) in soybean are genistin and daidzin, and their corresponding IFA are genistein and daidzein (**Fig. 1**). Soy isoflavones have been reported to have a variety of biological activities, including estrogenic (Shutt and Cox 1972), antioxidative (Kapiotis et al. 1997, Naim et al. 1976), antiosteoporotic (Anderson and Garner 1997, Ishida et al. 1998) and anticarcinogenic (Herman et al. 1995). Genistein in particular inhibits the proliferation of a variety of tumor cell lines in culture (Barnes 1995) and is thought to be the most effective isoflavone in cancer treatment and prevention.

In the last few years, several scientists have reported on the absorption, distribution, metabolism or excretion of soy isoflavone glucoside in humans (Franke and Custer 1996, Hutchins et al. 1995, Wang et al. 1994, Xu et al. 1995). King and Bursill (1998) investigated the concurrent patterns of plasma and urinary isoflavone excretion after consumption of soy meals containing IFG. Watanabe et al. (1998) evaluated more detailed pharmacokinetics of IFG in seven healthy Japanese men after consumption of a single dose of baked soybean powder containing IFG, and reported that more genistein than daidzein appeared in the blood circulation. King et al. (1996), in studies with rats on the pharmacokinetics of pure genistein or genistin that was contained in a soy extract, found that the plasma concentration of genistein in genistein-treated rats was more than two times higher than that in soy extract (genistin)-treated rats 2 h after the intake of an equal amount. It is generally thought that IFG are converted to the corresponding IFA by gut microflora or gut glucosidases, and then IFA are absorbed from the small intestine (Brown 1988, Day et al. 1998).

To date, however, no one has studied the difference be-

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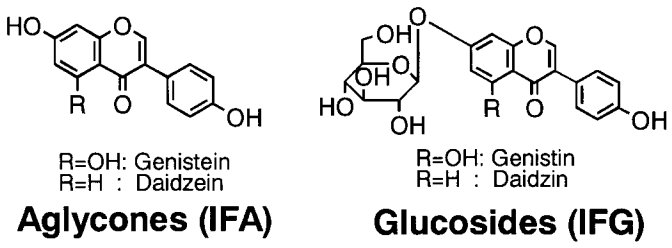

FIGURE 1 Soy isoflavones.

tween the absorption of IFA and IFG in humans. In this paper, we make the first report of this difference in absorption in eight healthy Japanese volunteers using the same molecular amounts of IFA and IFG.

MATERIALS AND METHODS

Chemicals. Daidzein (>99%), genistein (>99%) and H-5 sulfatase were purchased from Sigma Chemical (St. Louis, MO). Daidzin (>99%) and genistin (>99%) were purchased from Nacalai Tesque (Kyoto, Japan). Other chemicals were of analytical or HPLC grade.

Subjects. In low dose intake tests, the healthy volunteers were four men and four women between 31 and 58 y of age; body weight ranged from 51 to 85 kg and body height from 152 to 187 cm. In the high dose intake tests, the healthy volunteers were four men and four women between 38 and 57 y of age; body weight ranged from 54 to 80 kg and body height from 156 to 183 cm. In long-term intake tests, the subjects were eight men between 38 and 55 y of age with body weight of 58 to 82 kg and body height of 165 to 176 cm. The study design was approved by the ethics committee of the authors' laboratory, and informed consent of the subjects was obtained in writing.

Diet and blood sample collection. The studies consisted of four feeding days. In the single-intake tests, each study day was separated by a 2-mo washout period. In the low and high single-intake experiments, the participants were requested to avoid any soy foods, for example, miso (fermented soybean paste), natto (fermented soybean), tofu and soy protein products, from 3 d before the test until completion of the test. During the washout period, there were no diet restrictions.

IFA and IFG were provided in the form of tablets (**Table 1**). We used SoyAct (Kikkoman Corporation, Noda, Japan) as IFA or a soybean extract (prepared by the same company) as IFG. SoyAct is a fermented soybean extract containing 30% IFA (genistein, 43.63%; daidzein, 56.37%), saponin, sugar, protein and fat. The soybean extract contains ~40% IFG (genistin, 54.55%; daidzin, 45.45%), saponin, sugar, protein and fat. Concentration of isoflavones in those extracts was measured according to the method of Kudou et al. (1991a). The subjects took these tablets after breakfast (0930 h). Blood samples were collected in heparinized vacuum syringes by medical technologists before tablet intake (0900 h), and at 2 (1130 h), 4 (1330 h), 6 (1530 h) and 24 h (0930 h of the next day) after intake. Medical doctors consulted with the subjects about any health abnormalities during the tests.

In the long-term intake study, there was no diet restriction. The washout period was 4 mo between IFA intake and IFG intake. Subjects consumed tablets containing isoflavones (IFA 80 mg or IFG 130mg) after each meal, e.g., for IFA, 30 mg after breakfast, 20 mg after lunch and 30 mg after dinner. Blood samples were collected as above at 0, 2 and 4 wk (at 1100 h each day) after intake began.

In both single- and continuous-intake tests, blood samples were centrifuged at 2000 × *g* for 10 min at 4°C and the plasma was separated and stored at −20°C before analysis.

Isolation and identification of isoflavones in plasma. Plasma (50 μL) was added to 0.2 mol/L acetate buffer (pH 5.0, 50 μL) with 500 U H-5 sulfatase and incubated for 1 h at 37°C in a water shaking bath. Released aglycones were extracted with 0.9 mL methanol/acetic acid (100:5, v/v) with sonication and vortexing, and centrifuged at 5000 × *g* for 5 min at 4°C. The supernatant was diluted to double its quantity with 100 mmol/L lithium acetate in water, and the diluted samples were used for HPLC. Plasma concentration of isoflavones was measured according to the method of Piskula et al. (1999). The HPLC was carried out on an HPLC column (TSKgel ODS-80TS, 5 μm, 150 × 4.6 mm, TOSOH, Tokyo, Japan). The composition of the mobile phase for HPLC analysis was water/methanol/acetic acid (58:40:2, v/v/v) containing 50 mmol/L lithium acetate, and effluent was monitored by an amperometric electrochemical detector (ICA-3060, TOA, Tokyo, Japan) set at +950 mV.

Analysis of biochemical markers. Biochemical markers of the subjects, i.e., plasma triglyceride, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, γ-glutamyltranspeptidase, creatinine, blood urea nitrogen, total cholesterol, eosinocyte, erythrocyte and leukocyte, were analyzed at SRL Tokyo Medical (Chiba, Japan).

Statistics. Reported values represent means ± SD (n = 8). Statistical analysis was evaluated by paired t test to identify significantly different means; SigmaPlot for Windows Version 4.00 (SPSS, Chicago, IL) was used.

RESULTS

Low dose, single administration. With the consumption of IFA, plasma concentrations of genistein and daidzein reached their highest values 2 h after intake (**Fig. 2**). The concentration of genistein was higher ($P < 0.01$) than that of daidzein at each time point despite the similar intake of the two isoflavones (Fig. 2).

After IFG consumption, plasma concentrations of genistein and daidzein were highest 4 h after intake (Fig. 2), and, again, the concentration of genistein was higher ($P < 0.01$) at each time point. In these single-intake tests, the plasma concentration of either chemical after IFA intake was higher ($P < 0.05$)

TABLE 1

Intake of isoflavones in each test

		IFA[1]		IFG	
Intake type	Total isoflavone	Genistein	Daidzein	Genistin	Daidzin
	mg	*mmol*			
Low dose single IFA	30	0.048	0.062	—	—
Low dose single IFG	50	—	—	0.060	0.050
High dose single IFA	450	0.78	0.92	—	—
High dose single IFG	760	—	—	0.90	0.80
Long-term IFA	80	0.13	0.17	—	—
Long-term IFG	130	—	—	0.16	0.14

[1] IFA, isoflavone aglycones; IFG, isoflavone glucosides.

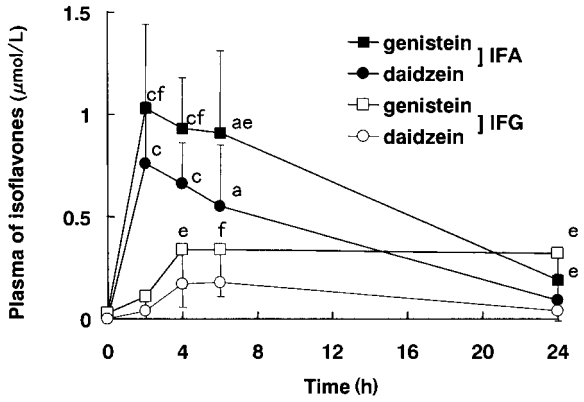

FIGURE 2 Plasma concentration of genistein and daidzein in four male and four female healthy Japanese subjects at 0, 2,4, 6 and 24 h after a single intake (0.11 mmol) of isoflavone aglycones (IFA) and isoflavone glucosides (IFG). Each point represents the mean ± SD, $n = 8$. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$ in comparison with genistein in IFA intake and that in IFG intake, and comparison with daidzein at 2, 4 and 6 h. $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.005$ in comparison with genistein and daidzein in IFA intake or in IFG intake at 2, 4 and 6 h.

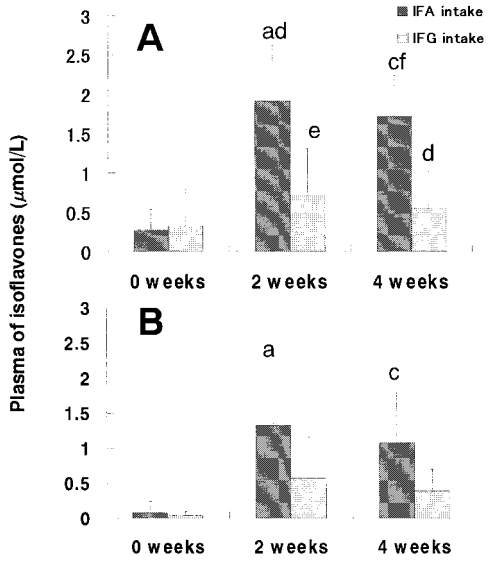

FIGURE 4 Plasma concentration of genistein (*panel A*) and daidzein (*panel B*) in eight healthy Japanese men during long-term (0.30 mmol/d) intake of isoflavone aglycones (IFA) or isoflavone glucosides (IFG) for 4 wk. Values are means ± SD, $n = 8$. Blood samples were collected as above at 0, 2 and 4 wk (before lunch) after intake began. Concentration during IFA intake was higher than during IFG intake; 2 wk ($^{a}P < 0.05$), and 4 wk ($^{c}P < 0.005$). Concentration of genistein was significantly higher than that of daidzein at 2 and 4 wk ($^{d}P < 0.05$; $^{f}P < 0.005$; $^{e}P < 0.01$; $^{d}P < 0.05$).

than after IFG intake at 2, 4, 6 h. All plasma biochemical markers measured were within the normal range after IFA intake (data not shown). No subjects complained of any health abnormalities.

High dose, single administration. When 1.7 mmol of IFA was consumed, plasma concentrations of genistein and daidzein were highest 4 h after intake (**Fig. 3**). Similar to after low IFA intake, the concentration of genistein was higher ($P < 0.01$) than that of daidzein at each time point despite the almost equal intakes.

Consumption of 1.7 mmol IFG resulted in the highest plasma concentrations of genistein and daidzein at 6 and 4 h after intake, respectively (Fig. 3). The concentration of genistein was higher ($P < 0.05$) at each time point despite nearly equal intakes. The concentrations after IFA intake were higher ($P < 0.05$) than those after IFG at 2, 4 and 6 h after ingestion (Fig. 3). The highest plasma concentration of either compound after IFA intake was more than five times higher than that after IFG (Fig. 3). Plasma total isoflavone concentration ratios after IFA intake relative to after IFG intake were 3.2, 6.6 and 4.3 at 2, 4 and 6 h after intake, respectively. All plasma biochemical markers were within the normal range after the intake of IFA. No subjects complained of any health abnormalities (data not shown).

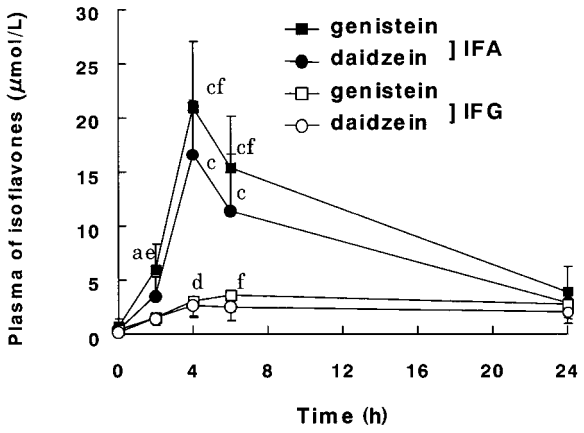

FIGURE 3 Plasma concentration of genistein and daidzein in four male and four female healthy Japanese subjects after a single intake (1.7 mmol) of isoflavone aglycones (IFA) and isoflavone glucosides (IFG). Each point represents the mean ± SD, $n = 8$. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$ in comparison with genistein in IFA intake and that in IFG intake, and comparison with daidzein at 2, 4 and 6 h. $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.005$ in comparison with genistein and daidzein in IFA intake or in IFG intake at 2, 4 and 6 h.

Long-term administration. The plasma concentration of genistein and daidzein during the consumption of IFA was more than twice as high as those of IFG at 2 ($P < 0.05$) and 4 wk ($P < 0.005$) after intake began (**Fig. 4**). The concentration of genistein was higher ($P < 0.05$) than that of daidzein at each time point during both IFA and IFG consumption. All plasma biochemical markers were within normal ranges after the intake of IFA. No subject complained of any health abnormalities (data not shown).

DISCUSSION

Among isoflavones, genistein has been reported to be a potent growth inhibitor in both MCF-7 breast cancer cells and MDA-468 cells (Peterson and Barnes 1991). Naik et al. (1994) reported that genistein inhibited the growth of MLL prostate cancer cells and PC-3 cells in a dose-dependent manner. Matsukawa et al. (1993) reported that genistein inhibited in a dose-dependent manner the growth of HGC-27 cells derived from human gastric cancer.

Using rats, King et al. (1996) studied the pharmacokinetics of pure genistein or genistin that was contained in a soy extract and found that the plasma concentration of genistein in genistein-treated rats was significantly higher than that in soy extract (genistin)-treated rats 2 h after intake. In our study, similar results were obtained in humans. IFG are very poorly absorbed from the gut compared with IFA, because of the higher hydrophilicity and greater molecular weight of IFG

(Brown 1988). It has been assumed that IFG have to be converted into IFA to be absorbed into the human body. Friend and Chang (1984) reported that glucosidases of intestinal microflora in the lower bowel could liberate the aglycones from the glucosides and promote their absorption. Day et al. (1998) reported that human gut tissues have a β-glucosidase capable of absorbing efficiently various naturally occurring isoflavonoid glucosides. We think that our results with humans support these reports in part. IFA was absorbed more quickly and in greater amounts than IFG. It is presumed that IFA are absorbed directly from the small intestine without being affected by gut microflora or gut glucosidases. In low and high dose intake tests, IFG administered required a longer time period to reach the highest isoflavone concentration in plasma than did IFA (Figs. 2, 3).

The time lags between the highest concentrations after IFA and IFG intake likely are attributable to the absorption of IFA from the stomach as found in the study with rats by Piskula et al. (1999). In plasma, the high dose took a longer time to reach the highest isoflavone concentration than did the low dose. The relative absorption ratio in the stomach may be higher with a low dose than with a high dose.

IFA are absorbed more efficiently than IFG. The intake of isoflavones in the high dose test was >15 times higher than that in the low dose test. The highest plasma concentration of total isoflavones in the high dose test was 21 times higher than that in the low dose test after IFA intake, whereas plasma concentration after the high dose test was only 12 times higher than after the low dose test when IFG was administered (Figs. 2, 3). These results suggest that IFG have to be converted to IFA by intestinal glucosidases to be absorbed into the human body, and that the conversion of IFG into IFA may be a rate-determining step in human absorption. The highest isoflavone concentrations in plasma after IFA intake were more than two times greater than those after IFG intake in the low dose, single administration, whereas at high administration, it was over five times greater (Figs. 2, 3).

In our study, as well as the studies by Watanabe et al. (1998) and King and Bursill (1998), the plasma concentration of genistein was higher than that of daidzein each time (Figs. 2, 3). Watanabe et al. (1998) reported that urinary daidzein excretion was much higher than that of genistein and that the half-life of genistein (8.4 h) in plasma was longer than that of daidzein (5.8 h) when subjects were fed baked soybean powder containing IFG. Our results also showed that genistein is higher and is elevated longer than daidzein in human plasma after IFA intake, perhaps allowing for certain pharmacological effects. Whole soy likely is superior to soy germ that is rich in daidzin and daidzein because of the larger amount of genistin and genistein it contains.

Soy isoflavones reportedly have estrogenic (Shutt and Cox 1972), antioxidative (Kapiotis et al. 1997, Naim et al. 1976), antiosteoporotic (Anderson and Garner 1997, Ishida et al. 1998) and anticarcinogenic (Herman et al. 1995) activities. They are expected to be effective against various conditions such as menopausal symptoms, coronary heart disease, osteoporosis and cancers. To obtain the desired effects, it is necessary to maintain a constant plasma concentration for a long period. In our long-term intake tests, the plasma concentrations of genistein and daidzein during the ingestion of IFA were >100% higher than those during IFG intake at 2 and 4 wk after the start of intake ($P < 0.05$) (Fig. 4), suggesting that IFA are more effective than IFG in maintaining the desired plasma concentrations. Moreover, we assume that the long-term intake of IFA or IFG may be completely safe because the subjects' plasma biochemical markers remained within normal ranges and no one complained of any unusual health problems.

In conclusion, the results of our single- and continuous-intake tests with IFA and IFG revealed the superior absorptivity of IFA in humans. Therefore, IFA are more useful than IFG in maintaining a high level of isoflavone concentration in plasma. Genistein is absorbed more efficiently than daidzein and a higher plasma concentration was maintained. We expect that our findings may be applicable to the treatment of certain diseases and conditions if the efficacious plasma concentrations of isoflavones are proved. Genistein-rich products such as fermented whole-soy foods and their extracts may be useful in preventing osteoporosis, menopausal symptoms, coronary heart disease and cancers.

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