Metabolism and disposition of isoflavone conjugated metabolites in humans after ingestion of *kinako*

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Dein, daidzein; Gein, genistein; ER, estrogen receptor; D-4’,7-diS, daidzein-4’,7-disulfate; D-7G-4’S, daidzein-7-glucuronide-4’-sulfate; HPLC, high-performance liquid chromatography; DAD, diode-array detector; G-7G-4’S, genistein-7-glucuronide-4’-sulfate; G-4’,7-diG, genistein-4’,7-diglucuronide; D-7-G, daidzein-7-glucuronide; G-7-G, genistein-7-glucuronide; D-4’,7-diG, daidzein-4’,7-diglucuronide; D-4’-G, daidzein-4’-glucuronide; G-4’-G, genistein-4’-glucuronide; D-7-S, daidzein-7-sulfate; D-4’-S, daidzein-4’-sulfate; G-7-S, genistein-7-sulfate; G-4’-S, genistein-4’-sulfate; G-4’,7-diS, genistein-4’,7-disulfate; Din, daidzin; Gin, genistin; SPE, solid-phase extraction; RSD, relative standard deviation; \( t_{\text{max}} \), time to maximum plasma concentration; \( C_{\text{max}} \), peak plasma concentration; AUC, area under the curve.
ABSTRACT:

Isoflavone aglycones daidzein (Dein) and genistein (Gein) are present primarily as glucuronides and sulfates in human plasma; however, very little is known about the plasma pharmacokinetics of isoflavone conjugates after soy ingestion. The aim of this study was to investigate metabolism and disposition after ingestion of kinako (baked soybean flour) by 10 volunteers of the isoflavone-conjugated metabolites glucuronide or sulfate or both. The quantifications of 16 metabolites in plasma and urine were performed by our previously reported high-performance liquid chromatography-UV-diode-array detector method. Plasma concentrations of total Dein and Gein metabolites reached maximum values of 0.64 ± 0.18 μmol/l at 4.7 ± 2.5 h and 1.58 ± 0.55 μmol/l at 5.4 ± 2.1 h, respectively. The area under the curve from 0 to 48 h demonstrated that D-7G-4’S (53.3%) was a major metabolite of Dein, and G-7G-4’S (54.0%) and G-4’,7-diG (26.6%) were major metabolites of Gein in plasma. The compositions of isoflavone metabolites in urine and plasma differed greatly. About half of the 48-hour urinary excretion of total Dein metabolites consisted of Dein-7-glucuronide. The total amounts of Gein-7-glucuronide and Gein-4’-glucuronide were half the total amount of the urinary Gein metabolites. Excretion into urine of D-7G-4’S and G-7G-4’S accounted for only 16% each of the total Dein and Gein
metabolites, respectively. The plasma and urine profiles of 16 metabolites of Dein and Gein demonstrate the involvement of desulfation and deglucuronidation of the conjugated metabolites D-7G-4’S, G-7G-4’S and G-4’,7-diG in the process of renal excretion.
Introduction

The isoflavonoid phytoestrogens daidzein (Dein) and genistein (Gein) are thought to have many beneficial properties, particularly due to their effects on osteoporosis, the cardiovascular system, and breast and prostate cancers in humans and animals (Arjmandi et al., 1996; Liu et al., 2009; Taku et al., 2010; Martin et al., 2008; Messina and Wood, 2008; Bektic et al., 2005).

The aglycones of isoflavonoids absorbed through the intestine and their unconjugated metabolites are converted to either their glucuronide and sulfate conjugates, or to mixed conjugates, as phase II metabolites. The conjugated metabolites are then excreted into urine as mono- and diglucuronides, mono- and disulfates, and sulfoglucuronides at either or both of the 4’ or 7 positions on the isoflavone ring (Adlercreutz et al., 1995). Small amounts of intact aglycones have also been detected in plasma (Zhang et al., 2003).

Isoflavonoids have been well studied and possess numerous biological activities. It is known that aglycones such as Dein and Gein, as well as equol, a metabolite of Dein, have biological effects such as a much higher affinity for estrogen receptor (ER)-β than ER-α (Kuiper et al., 1998; Nikov et al., 2000), inhibitory activity against topoisomerase II and antioxidant activity (McCue and Shetty, 2004; Arora et al., 1998). In addition,
many reports have been published describing pharmacological or physiological effects of isoflavone conjugated metabolites, such as the inhibitory effect of Dein-4’,7-disulfate (D-4’,7-diS) on sterol sulfatase in hamster liver microsomes (Wong and Keung, 1997), the stimulatory effect of Dein-7-glucuronide-4’-sulfate (D-7G-4’S) on the growth of MCF-7 cells (Kinjo et al., 2004), the hypotensive and vasodilator effects of Dein sulfates in rats (Cao et al., 2006), and the weak estrogenic activity of Dein and Gein glucuronides (Zhang et al., 1999).

Pharmacokinetic data on isoflavones and their conjugated metabolites have generally been estimated by measuring the free aglycones obtained after enzymatic hydrolysis (reviewed in Nielsen and Williamson, 2007). In order to assess metabolism and disposition of isoflavone conjugated metabolites, a direct measurement of the metabolites without hydrolysis is required, since the majority of isoflavones are present as glucuronidated and sulfated forms in human plasma and urine. The pharmacokinetics of isoflavones and their conjugated metabolites are still obscure.

“Kinako” is “baked soybean flour”, and it is used in many products, particularly in Japanese-style confections and as a “kinako beverage” suspended in cow milk on the traditional custom of consuming soy products for health. We previously synthesized 12 conjugated metabolites of Dein and Gein and isolated two compounds from human
urine after ingestion of *kinako* for use as the authentic standard, and we then developed a high-performance liquid chromatography (HPLC)-UV-diode-array detector (DAD) method for simultaneous determination of 16 isoflavone metabolites (Fig. 1) in plasma (Hosoda et al., 2010a). By using this method, we found that D-7G-4’S, Gein-7-glucuronide-4’-sulfate (G-7G-4’S) and Gein-4’,7-diglucuronide (G-4’,7-diG) were the major metabolites of Dein and Gein in humans. The aim of this study was to evaluate the metabolism and disposition of isoflavone-conjugated metabolites by directly measuring 16 conjugated metabolites of Dein and Gein in plasma and urine after ingestion of *kinako* by 10 healthy volunteers.
Materials and Methods

Chemicals. Daidzein (7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 4’,7-dihydroxyisoflavone) was purchased from LC Laboratories (Woburn, MA). Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 4’,5,7-trihydroxyisoflavone) and luteolin-3’,7-di-O-glucoside were purchased from Extrasynthese (Genay, France). Daidzein-7-glucuronide (D-7-G), genistein-7-glucuronide (G-7-G), daidzein-4’,7-diglucuronide (D-4’,7-diG) and G-4’,7-diG were synthesized in our laboratory according to the method of Needs and Williamson (2001) (Hosoda et al., 2008; Hosoda et al., 2010b). D-7G-4’S and G-7G-4’S were synthesized in our laboratory according to the method of Soidinsalo and Wäähälä (2007) (Hosoda et al., 2010b). Daidzein-4’-glucuronide (D-4’-G) and genistein-4’-glucuronide (G-4’-G) were isolated from human urine (Hosoda et al., 2008). Daidzein-7-sulfate (D-7-S), daidzein-4’-sulfate (D-4’-S), D-4’,7-diS, genistein-7-sulfate (G-7-S), genistein-4’-sulfate (G-4’-S) and genistein-4’,7-disulfate (G-4’,7-diS) were synthesized in our laboratory (Nakano et al., 2004). Phosphoric acid (99.999%) was purchased from Sigma-Aldrich (St. Louis, MO). Ammonia solution (28%) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals and solvents were of analytical grade and were used without further
purification. Stock standard solutions of each compound were prepared by dissolving the appropriate amount of each compound in methanol followed by dilution with methanol-water (50:50, v/v) as working standard solutions, except for Dein. The stock standard solution of Dein was prepared by dissolving the appropriate amount of Dein in ethanol followed by dilution with ethanol-water (50:50, v/v).

**Kinako Isoflavones.** Kinako was purchased from a retail store. Ten grams of kinako containing 5.5 mg (13.2 μmol) of daidzin (Din), 9.2 mg (21.2 μmol) of genistin (Gin), 6.1 mg (23.9 μmol) of Dein and 11.1 mg (41.0 μmol) of Gein were suspended in 150 ml of cow milk. The malonyl and acetyl glycosides were not contained in kinako powder.

**Study Design.** Ten healthy Japanese volunteers (5 men and 5 women) aged 21-55 (mean ± SD, 35.0 ± 11.7) years, with a body mass index of 18.5-24.8 (mean ± SD, 21.3 ± 2.5) kg/m², participated in the study. The study was approved by Kyorin University, School of Health Sciences Human Subjects Review Board. Written informed consent was obtained from all subjects. The volunteers did not have allergies to soybean or cow milk. They did not ingest isoflavone-containing foods from one week before the test until completion of the study. Blood samples (7.0 ml each) were collected from a median cubital vein into evacuated tubes containing Na₂EDTA just before (blank) and at 1, 2, 4, 6, 8, 10, 24, and 48 h after ingestion of 10 g of kinako and were immediately
centrifuged (2500 rpm, 10 min). The plasma samples were stored at -20°C until analysis. Urine samples were collected two hours before (blank) and at 1, 2, 4, 6, 8, 10, 24, 36, and 48 h after ingestion. The urine samples (ca. 30 ml) were stored at -20°C and analyzed within one week.

**Analytical Methods.**

1) **HPLC-UV-DAD analysis of plasma isoflavone metabolites.**

The determinations of isoflavone metabolite levels in plasma were performed according to the previously developed HPLC-UV-DAD method (Hosoda et al., 2010a). The metabolites in plasma were extracted by solid-phase extraction (SPE) using an Oasis HLB cartridge (3 cm³, 60 mg of packing; Waters, Milford, MA). HPLC-UV-DAD analyses were performed using a Hydrosphere C18 column (100 × 4.6 mm i.d., particle size 3 μm; YMC Co. Ltd., Kyoto, Japan) with luteolin-3’,7-di-O-glucoside as an internal standard.

2) **HPLC-UV analysis of urinary isoflavone metabolites.**

**Extraction from urine.** Distilled water was added to dilute the volumes of the collected urine samples 1/4000 within each interval, and the total volumes of the diluted urine samples were adjusted to 1.0 ml. The subsequent sample analyses were performed according to the previously developed SPE method (Hosoda et al., 2010a).
Luteolin-3’7-di-O-glucoside (510.0 ng) was added as an internal standard to the diluted urine samples (1.0 ml). The diluted urine samples were further diluted by adding 2.0 ml of 50 mM phosphoric acid solution (pH 2.0). An Oasis HLB cartridge (3 cm³, 60 mg of packing; Waters, Milford, MA) was placed on a vacuum manifold and activated with 2.5 ml of methanol, followed by 2.5 ml of 50 mM phosphoric acid solution. The urine sample diluted with a phosphoric acid solution was then loaded onto the cartridge at a flow rate of 0.2 ml/min. The cartridge was washed with 5.0 ml of methanol-50 mM phosphoric acid solution (20:80, v/v, pH 2.0). After purging the cartridge with air, the sample was eluted with 2.5 ml of methanol-28% ammonia solution (95:5, v/v, pH 10.0) at a flow rate of 0.2 ml/min. After the eluate was evaporated at 40°C in vacuo, the residue was dissolved in 1.0 ml of methanol and the solution was filtered through an HLC-Disk filter (pore size 0.45 μm) (Kanto Chemical, Tokyo, Japan). The filtrate was transferred to a spitz tube with a ground-glass joint and evaporated to dryness at 40°C in vacuo. The residue was dissolved in 50 μl of methanol with vortex mixing for 30 s and then 150 μl of 10 mM ammonium acetate solution were added with vortex mixing for 30 s. A 20-μl aliquot of the solution was subjected to HPLC.

**HPLC-UV analysis.** HPLC-UV analyses were performed on an LC-2000 Plus liquid chromatograph system (Jasco, Tokyo, Japan) equipped with a model PU-2089 Plus
pump, a model UV-2075 Plus UV-visible detector and a model CO-2067 Plus column oven. Data processing was performed with a model Sic chromatocorder 21 (System Instruments, Tokyo, Japan). Chromatographic separation was performed on a Hydrosphere C18 column (150 × 4.6 mm i.d., particle size 3 μm; YMC Co. Ltd., Kyoto, Japan) and a guard cartridge (23 × 4.0 mm i.d.) of the same material. Elution was performed using a solvent system comprised of 10 mM ammonium acetate solution (solvent A) and acetonitrile mixed using a linear gradient. Gradient elution (0-1.5 min, 95% solvent A; 1.5-9 min, linear change from 95 to 88% solvent A; 9-13 min, 88% solvent A; 13-15.5 min, linear change from 88 to 84.8% solvent A; 15.5-19.5 min, linear change from 84.8 to 83.8% solvent A; 19.5-40.5 min, linear change from 83.8 to 62.8% solvent A; 40.5-50.6 min, linear change from 62.8 to 95% solvent A) was performed with a 1.3 ml/min constant flow rate at 40°C. The column was equilibrated for 13 min before any subsequent injections. The UV detection wavelength was set at 250 nm.

**Extraction recovery.** Extraction recoveries of isoflavone metabolites by SPE were obtained from blank urine samples analyzed before and after the addition of known amounts of these analytes. The extraction recovery (mean ± SD, n = 4) for 16 isoflavone metabolites was 97.6 ± 4.3%. The absolute recovery of the internal standard
(luteolin-3’,7-di-O-glucoside) from urine was 98.4%.

*Accuracy and precision.* Inter- and intra-assay accuracy and precision were determined by assaying in duplicate six preparations of blank human urine containing known amounts of isoflavone metabolites and a fixed amount (510.0 ng) of luteolin-3’,7-di-O-glucoside as the internal standard. Inter-assay relative standard deviation (RSD) for isoflavone metabolites ranged from 0.4-9.7%. Intra-assay RSD for isoflavone metabolites ranged from 0.8-3.8%. The sixteen isoflavone metabolites in urine considered in this study were stable for at least one week at -20°C [88.9% (Dein)-102.9% (G-4’S)].
Results

Plasma Profile of Isoflavone Metabolites. The mean plasma concentrations of Dein, Gein and their metabolites at 1, 2, 4, 6, 8, 10, 24, and 48 h in 10 subjects after ingestion of 10 g of kinako are shown in Fig. 2. The time to maximum plasma concentration ($t_{\text{max}}$), peak plasma concentration ($C_{\text{max}}$) and area under the curve (AUC) from 0 to 48 h of Dein, Gein and their metabolites are shown in Table 1. AUC values were calculated using the trapezoidal rule. Large inter-individual differences were observed in $t_{\text{max}}$, $C_{\text{max}}$ and AUC values for total Dein metabolites in the subjects: a range of 1.0-10.0 h (10 times; RSD, 53.2%; mean, 4.7 h) for $t_{\text{max}}$, 0.43-1.06 μmol/l (2.5 times; RSD, 28.2%; mean, 0.64 μmol/l) for $C_{\text{max}}$, and 7.05-20.70 μmol•h/l (2.9 times; RSD, 37.4%; mean, 11.70 μmol•h/l) for AUC, respectively. There were also large inter-individual differences in $t_{\text{max}}$, $C_{\text{max}}$ and AUC values for total Gein metabolites: a range of 2.0-8.0 h (4.0 times; RSD, 38.9%; mean, 5.4 h) for $t_{\text{max}}$, 0.81-2.55 μmol/l (3.1 times; RSD, 34.8%; mean, 1.58 μmol/l) for $C_{\text{max}}$, and 16.02-50.92 μmol•h/l (3.2 times; RSD, 33.7%; mean, 31.05 μmol•h/l) for AUC, respectively. The mean AUC value for the total Gein metabolites (31.05 μmol•h/l) was 2.7 times that for the total Dein metabolites (11.70 μmol•h/l). For all subjects, the AUC value for the total Gein metabolites was 1.5-3.9 times as large as that obtained for the Dein metabolites. The mean AUC value for
D-7G-4’S as a percentage of the total Dein metabolites was 53.3%. The AUC value for D-7G-4’S was the largest among the total Dein metabolites for all subjects. On the other hand, the mean AUC value for G-7G-4’S was ca. half (54.0%) of that found for the total Gein metabolites. However, in three subjects, the AUC for G-4’,7-diG was greater than that for G-7G-4’S. The mean AUC value for G-4’,7-diG comprised 26.6% of the total Gein metabolites secondary to G-7G-4’S.

**Urine Profile of Isoflavone Metabolites.** Quantification of the urinary isoflavone metabolites was performed according to our previously developed HPLC-UV-DAD method for plasma analysis. Fig. 3 shows the mean urinary excretion rates of Dein, Gein and their metabolites during the intervals 0-1, 1-2, 2-4, 4-6, 6-8, 8-10, 10-24, 24-36, and 36-48 h in 10 subjects after ingestion of 10 g of *kinako*. The mean excretion rates for total Dein and Gein metabolites gradually increased to reach a peak at 6-8 h and 4-6 h, respectively. The excretion rate of D-7-G was greater than that of other Dein metabolites during all of the time intervals except 24-36 and 36-48 h, and it reached a peak at 4-6 h. The mean urinary excretion of each metabolite within 48 h is shown in Table 2. The urinary excretion of D-7-G ranged between 7.47 to 18.20 μmol (mean, 12.86 μmol) and comprised 48.1% of the total Dein metabolites (mean, 26.73 μmol), which was the greatest percentage found for all subjects (31.8-68.0%). The excreted
amounts of G-7-G (5.23 μmol) and G-4’-G (4.95 μmol), were greater than that of the other Gein metabolites, comprising 28.5% and 27.0%, respectively, of their total (mean, 18.34 μmol). In one exceptional case, G-4’,7-diG excreted into the urine of a male subject comprised 50.1% of the total Gein metabolites; in this case, the AUC of G-4’,7-diG corresponded to 51.1% of the total Gein metabolites.

The recovery of each metabolite in urine (% of dose) is shown in Table 2. The recoveries of total Dein and Gein metabolites were between 44.3% and 96.3% (mean, 72.1%), and between 11.9% and 61.0% (mean, 29.5%), respectively. The mean recovery of total Dein metabolites was 2.4 times that of the total Gein metabolites, and this phenomenon was observed for all subjects (1.4-4.4 times).
Discussion

Until now, most investigators have made an effort to describe the pharmacokinetics of isoflavone, especially the total Dein and Gein, after ingestion of soybean products containing primarily the isoflavonoid glycosides such as Din and Gin. Pharmacokinetic data such as $C_{\text{max}}$, $t_{\text{max}}$, AUC and elimination half-life were obtained by measuring released aglycones (Dein and Gein) after hydrolysis of the conjugated metabolites (reviewed in Nielsen and Williamson, 2007). These results showed that the bioavailability of Gein is much greater than that of Dein (Setchell, 2001; Larkin et al., 2008; reviewed in Prasain and Barnes, 2007). Although the pharmacokinetic data such as $C_{\text{max}}$, $t_{\text{max}}$, AUC and elimination half-life of the glucuronide and sulfate conjugates of Gein and Dein were reported, the data for each conjugate were not known. It turned out that there are significant differences in the pharmacokinetics of sulfate and glucuronide conjugates of isoflavones (Shelnutt et al., 2002). We present here the first report investigating the metabolism and disposition of conjugated metabolites of Dein and Gein, which are classified according to the type of conjugates and the conjugation position (s) (4’ or 7) on the isoflavone skeleton.

The mean $C_{\text{max}}$ and AUC of the total Gein metabolites were 2.5 times and 2.7 times those of the Dein metabolites, respectively. The ratio of AUC of Gein to that of Dein
was higher than the composition ratio (1.7 times) expected from the amounts of Gein isoflavones (Gein and Gin-derived Gein) and Dein isoflavones (Dein and Din-derived Dein) contained in *kinako*. On the other hand, the recovery of total Dein metabolites in urine (72.1%) was 2.4 times that of the total Gein metabolites (29.5%). The higher AUC of Gein relative to Dein is consistent with a scenario in which Gein and its conjugated metabolites are more extensively excreted in bile than in urine, and undergo enterohepatic circulation thereby contributing to persistence of Gein in the body. Taking into consideration that total fecal excretion of isoflavones is typically less than 5% (Larkin et al., 2008), the fractions of Gein and Gein-derived metabolites absorbed from the gastrointestinal tract should be much greater than that of Dein. Gein may be converted to unknown metabolites and then excreted in urine.

The AUC from 0 to 48 h demonstrated that D-7G-4’S (53%) was a major metabolite of Dein, and G-7G-4’S (54%) and that G-4’,7-diG (27%) were major metabolites of Gein in plasma. Several investigators have reported that the conjugates may have biological activity themselves, or they may be precursors of biologically active compounds at or within target cells. Therefore, these conjugated metabolites could be key compounds responsible for pharmacological and medicinal properties of isoflavone, either directly or indirectly.
The mixed conjugate metabolites with glucuronide and sulfate, D-7G-4’S and G-7G-4’S, comprised 53% and 54% of the total conjugated metabolites of Dein or Gein respectively, making them the major metabolites in plasma, whereas the 48-hour urinary excretion of D-7G-4’S and G-7G-4’S were both only 16% of the total urinary excretion of Dein or Gein metabolites, respectively (Fig. 4A, 4B). D-7-G and G-7-G comprised only 12% and 6% of the total Dein or Gein metabolites in plasma, respectively, and these values increased to 48% or 29% of the total Dein or Gein metabolites in urine, respectively. Therefore, it was suggested that as a result of desulfation, D-7G-4’S and G-7G-4’S changed to D-7-G and G-7-G, respectively. G-4’,7-diG comprised 27% of the total conjugated metabolites of Gein, and the metabolite decreased to 20% of the total urinary excretion of Gein. Therefore, it was suggested that G-4’,7-diG was converted to G-4’-G by deglucuronidation at the 7-position glucuronic acid of G-4’,7-diG. Significant differences in AUCs and urinary excretion were not observed in the plasma and urinary profiles of other conjugates (D-4’,7-diG, D-4’-G, D-7-S, G-7-S, D-4’-S, and G-4’-S) and aglycones (Dein and Gein). D-4’,7-diS and G-4’,7-diS were not detected in the plasma and urine of any of the subjects. We confirmed the presence of equol in four of ten subjects by HPLC analyses of urine samples obtained by the enzymatic hydrolysis. Although one subject produced nearly equivalent amount of
equol to Dein, other three subjects produced only a small amount of equol (unpublished data). A more detailed study on the analysis of equol in plasma and urine is required and will be reported in due course.

The double conjugated metabolites D-7G-4’S, G-7G-4’S and G-4’,7-diG were found to be the most abundant compounds in plasma after ingestion of *kinako*. On the other hand, it was previously reported that D-7-G and D-4’-G were major metabolites of Dein found in urine (Doerge et al., 2000; Clarke et al., 2002; Shelnutt et al., 2002). Our data also showed that the monoglucuronides D-7-G, G-7-G and G-4’-G were the most abundant conjugated metabolites in urine. That is to say, it turned out that the highly polar double conjugates of isoflavones in plasma do not readily undergo renal excretion. As far as we aware, this is the first report to suggest that desulfation or deglucuronidation of the conjugated metabolites D-7G-4’S, G-7G-4’S and G-4’,7-diG during renal excretion is possible.
Authorship Contributions

Participated in research design: Kaori Hosoda, Takashi Furuta, and Kazuo Ishii.

Conducted experiments: Kaori Hosoda and Kazuo Ishii.

Performed data analysis: Kaori Hosoda, Takashi Furuta, and Kazuo Ishii.

Wrote or contributed to the writing of the manuscript: Kaori Hosoda, Takashi Furuta, and Kazuo Ishii.
References


Kinjo J, Tsuchihashi R, Morito K, Hirose T, Aomori T, Nagao T, Okabe H, Nohara T,


Setchell KDR (2001) Soy isoflavones – benefits and risks from nature’s selective


Footnotes

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Figure legends

Fig. 1 Structures of daidzein (Dein) and genistein (Gein) showing the 7 and 4’ positions where either glucuronidation (G) or sulfation (S) can occur, and their conjugated metabolites considered in this study.

Fig. 2 Plasma concentrations of Dein, Gein and their conjugated metabolites from 10 subjects. Each bar represents the mean ± SD.

Fig. 3 Urinary excretion rates of Dein, Gein and their conjugated metabolites from 10 subjects. Each bar represents the mean ± SD. The total bar indicates the total urinary excretion rate of isoflavone metabolites.

Fig. 4 Percentages of each isoflavone metabolite among the total isoflavone metabolites in plasma and excreted into urine, measured by AUC and 48-hour urinary excretion, respectively. (A) Dein metabolites; (B) Gein metabolites.
Table 1 Pharmacokinetic variables for Dein, Gein and their metabolites in 10 subjects

<table>
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<td>20.2</td>
<td>6.4 ± 2.5</td>
<td>38.4</td>
<td>0.85 ± 0.39</td>
<td>46.2</td>
<td>16.78 ± 8.03</td>
<td>47.8</td>
<td>5.4 ± 2.1</td>
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<td>D-7-G</td>
<td>6.1 ± 2.6</td>
<td>42.6</td>
<td>0.09 ± 0.04</td>
<td>46.2</td>
<td>1.72 ± 1.22</td>
<td>70.8</td>
<td>5.0 ± 1.7</td>
<td>34.0</td>
<td>0.11 ± 0.04</td>
<td>39.9</td>
<td>1.85 ± 1.03</td>
<td>55.6</td>
<td>3.0 ± 1.8</td>
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<td>54.7</td>
<td>0.10 ± 0.07</td>
<td>72.6</td>
<td>1.63 ± 1.70</td>
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<td>2.6 ± 1.4</td>
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<td>79.1</td>
<td>0.09 ± 0.03</td>
<td>32.0</td>
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<td>61.5</td>
<td>1.7 ± 1.3</td>
<td>73.6</td>
<td>0.18 ± 0.11</td>
<td>60.5</td>
<td>1.58 ± 1.00</td>
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<td>1.7 ± 1.3</td>
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<td>AUC</td>
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<td>20.2</td>
<td>6.4 ± 2.5</td>
<td>38.4</td>
<td>0.85 ± 0.39</td>
<td>46.2</td>
<td>16.78 ± 8.03</td>
<td>47.8</td>
<td>5.4 ± 2.1</td>
<td>38.9</td>
<td>1.58 ± 0.55</td>
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<sup>t<sub>max</sub></sup>, time to maximum plasma concentration; <sup>C<sub>max</sub></sup>, peak plasma concentration; AUC, area under the curve.
Table 2 Urinary excretion and recoveries of Dein, Gein and their metabolites from 10 subjects

<table>
<thead>
<tr>
<th></th>
<th>Excretion (μmol)</th>
<th>( % of dose )</th>
<th>Recovery (μmol)</th>
<th>( % of dose )</th>
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<tr>
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<td>Mean ± SD</td>
<td>RSD</td>
<td>Mean ± SD</td>
<td>RSD</td>
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<td>Dein</td>
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<td>1.0 ± 0.9</td>
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<td>8.8 ± 7.0</td>
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<td>11.6 ± 8.4</td>
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<td>1.5 ± 0.8</td>
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<td>Total</td>
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<td>72.1 ± 16.4</td>
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<td>63.7</td>
<td>5.9 ± 3.8</td>
<td>63.8</td>
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<tr>
<td>G-4',7-diS</td>
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<tr>
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<td>G-7-G</td>
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<td>8.0 ± 5.2</td>
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<td>0.34 ± 0.17</td>
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<td>0.5 ± 0.3</td>
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</table>

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Fig. 1
(A) Dein metabolites

(B) Gein metabolites

Fig. 2
(A) Dein metabolites

(B) Gein metabolites

Fig. 3
Fig. 4