Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans

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ABSTRACT The aim of this study was to determine the pharmacokinetics and urinary excretion patterns of the soy isoflavones daidzein and genistein in humans. Six healthy men with a mean age of 37 y and a mean body mass index (in kg/m²) of 24 consumed a soybean flour–based meal on two occasions ∼6 d apart. Blood samples and total urine were collected at intervals for the measurement of daidzein and genistein with HPLC. Isoflavone concentrations rose slowly and reached maximum values of 3.14 ± 0.36 µmol/L at 7.42 ± 0.74 h for daidzein and 4.09 ± 0.94 µmol/L at 8.42 ± 0.69 h for genistein. Elimination half-lives were 4.7 ± 1.1 and 5.7 ± 1.3 h for daidzein and genistein, respectively. The slow increase in plasma concentrations is consistent with the facilitation of absorption by hydrolysis in the small and large intestines of the glycosidic forms of the isoflavones present in soybean-containing foods to their corresponding aglycones. The rate of urinary excretion of daidzein was greater than that of genistein throughout the postmeal period, with mean recoveries of 62 ± 6% and 22 ± 4% (P < 0.001) for daidzein and genistein, respectively. However, the ratio of the areas under the plasma concentration versus time curves for daidzein and genistein was equal to the ratio of the concentrations of the respective isoflavones in the soy meal. It is concluded that the bioavailabilities of daidzein and genistein are similar, not withstanding the difference in urinary excretion.

INTRODUCTION

The role of dietary flavonoids in the prevention of several chronic diseases is the subject of intense research interest and the soy isoflavones have been the focus of particular attention (1–3). One important component of the evaluation of the potential importance of flavonoids in human health is an understanding of the absorption, distribution, metabolism, and excretion of these substances after their ingestion. However, although some information on the pharmacokinetics and bioavailability of some flavonoids in humans has been reported (4–7), much less is known about the isoflavones and the need for more research was highlighted recently (8).

With regard to the isoflavones, we reported previously on the pharmacokinetics and bioavailability of daidzein (7,4'-dihydroxy-isoflavone) and genistein (5,7,4'-trihydroxy-isoflavone) in rats (9, 10). In these studies we showed differences between the aglycone and conjugated forms of genistein and between the conjugates of daidzein and genistein administered as a soy extract. In addition, the pharmacokinetics of the synthetic isoflavone aglycone, ipriflavone (7-isopropoxy-isoflavone), were also reported for humans (11), but relatively little is known about the glycosidic forms of the isoflavones found in nature. Thus, although several studies have examined urinary excretion of daidzein and genistein (12–17), plasma concentrations were reported only at 6.5 and 24 h after single (14, 15) or multiple (13) soy meals. In the present study, therefore, we compared the absorption, pharmacokinetics, and urinary excretion of daidzein and genistein in detail over 35 h after a single soybean flour–based meal to provide further information about the possible importance of these substances to human health.

SUBJECTS AND METHODS

Subjects

The subjects were six healthy male volunteers (five white and one Asian) aged 21–48 y (mean: 37 y) with a mean body mass index (in kg/m²) of 24 (range: 19–39). The protocol was approved by the CSIRO Division of the Human Nutrition Human Ethics Committee and was fully explained to all volunteers, who gave their informed written consent.

Study design

The study was divided into two phases spaced ∼6 d apart to provide a protocol easy to manage without the capacity to obtain blood samples over a continuous 24-h postmeal period. Subjects were instructed to follow a soy-free diet for 1 wk before phase 1 of the study. Phase 1 was designed to determine the time required to reach maximum plasma isoflavone concentrations. After an overnight fast, subjects consumed a soy meal over a period of a few minutes at 0800. The meal consisted of debittered soy flour (Lowan Whole Foods, Nhill, Victoria, Australia) that had been heat treated by the manufacturer at 105 °C with...
moist heat during processing. The flour was suspended in 300–400 mL cow milk to provide 0.84 g flour/kg body wt, to which was added powdered chocolate flavoring to taste. This provided 2.7 μmol daidzein and 3.6 μmol genistein/kg body wt. Meals or snacks, excluding soy products, were permitted throughout the 8-h postmeal period, but generally only liquids were consumed until ≈5 h after the meal.

Ten-milliliter blood samples were taken from a medial cubital vein into evacuated tubes containing K₂EDTA (final concentration: 1.8 g/L) before and 1, 2, 4, 6, and 8 h after consumption of the soy meal. Blood samples were centrifuged immediately (15 min at 4000 × g and 5°C) and duplicate 200-μL aliquots were taken into screw-capped glass vials and stored at −20°C until assayed within 4 wk. Urine was collected into bottles for 24 h immediately before the study to provide baseline values, and for the intervals 0–1, 1–2, 2–4, 4–6, and 6–8 h after the meal. Preservation (1 g ascorbic acid + 1 g sodium azide) was added to the urine samples from the other collection periods, but in all cases the samples were stored on ice for <1 h and duplicate 200-μL aliquots for isoflavone assay were immediately taken into sealed glass vials and stored at −20°C until assayed within 5 wk.

Phase 2 of the study was designed to determine the elimination half-life. The meal was consumed at 2200 and blood samples were collected 11, 12, 14, 16, 18, and 35 h after the meal. Urine samples were collected over the periods 0–11, 11–12, 12–14, 14–16, 16–18, and 18–35 h after the meal. Preservative was provided in the collection bottles for the 0–11-h and 18–35-h samples. Aliquots of plasma and urine for isoflavone assay were also taken and stored as for phase 1.

For one volunteer, blood samples were collected 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, and 24 h after the 0800 meal and 0.5, 1, 2, 4, 6, 8, 11, 12, 14, 16, 18, and 35 h after the 2200 meal to validate the two-phase design. Samples obtained during the period 1–8 h after the 0800 meal and 11–35 h after the 2200 meal were taken from a medial cubital vein by using evacuated collection tubes and processed immediately as detailed above. Corresponding urine samples were also dispensed as aliquots immediately as detailed above. The remaining blood samples were collected by the volunteer from an indwelling catheter inserted in a cephalic vein. For these samples 5 mL blood was removed and discarded before collection of a further 10 mL, which was kept on ice. Total urine samples for the corresponding intervals were also collected and stored on ice. Blood and urine samples were transferred to the laboratory at 0800 the next morning for immediate processing.

Analytic methods

Genistein and β-glucuronidase/sulfatase (product #G0876) were obtained from Sigma-Aldrich, Castle Hill, Australia. Daidzein was purchased from Research Biochemicals International, Natick, MA. Equol was purchased from Plantech (UK), Reading, United Kingdom. All other reagents were analytic grade or better. The concentrations of genistein, daidzein, and equol in HPLC standard solutions were determined by using the extinction coefficients as described previously (9): ε₃20 37 260, ε₃25 27 542, and ε₃24 310 400, respectively.

The free (aglycone) and total (aglycone + glycoside) isoflavone contents of the soy flour were measured by using the method of Pettersson and Kiessling (19) with minor modification as described previously (9). The total genistein content was 4.44 μmol/g and the daidzein content was 3.27 μmol/g, of which <3% were in the respective free forms.

Isoflavone conjugates in plasma and urine were hydrolyzed, extracted, and assayed by using HPLC with electrochemical detection as described previously (9), with the following minor modifications. Three extractions with 0.5 mL diethyl ether were used to maximize recovery. Glass vials (Chromacol Ltd, Hertfordshire, United Kingdom) were used instead of plastic tubes for incubations and ether extractions to prevent extraction from plastic tubes of a substance that we showed would otherwise interfere with HPLC measurement of daidzein. HPLC conditions and equipment were as described previously (9), except that the mobile phase consisted of 50:50:1 methanol:0.1 mol ammonium acetate/L, pH 4.6:25 mmol EDTA/L (by vol). Typical HPLC profiles of plasma and urine samples collected before and after the consumption of the soy meal are shown in Figure 1.

Recoveries for daidzein, genistein, and equol, as determined from quadruplicate estimates from plasma and urine samples containing known amounts, ranged from 72% to 78% with SEs <5%. All results were corrected for the individual recovery rates.

Statistics

The significance of differences was determined by using Student’s t test (INSTAT; GraphPad Software, San Diego). A P value <0.05 was considered significant.

RESULTS

Plasma concentrations of daidzein and genistein from the single subject in whom concentrations were continuously monitored for ≥24 h after a soy meal at 0800 and at 2200 6 d later are shown in Figure 2. There was a close similarity between the pro-

![FIGURE 1. Typical HPLC profiles of premeal (A, C) and postmeal (B, D) samples of urine (A, B) and plasma (C, D). d, daidzein; g, genistein. “Electrode response” refers to the electrical response of the HPLC electrochemical detector.](https://academic.oup.com/ajcn/article-abstract/67/5/867/4666162/87087465162)
files obtained from the two study phases, confirming the validity of the two-phase experimental design. Note that plasma isoflavones were detectable as early as 0.5 h after the 2200 meal. The validity of the two-phase design is also supported by the data shown in Figure 3, which shows the mean plasma isoflavone profile of the six volunteers. In particular, there was no evidence of any discontinuity in the profile between 8 and 11 h. The mean ratio of the areas under the respective curves for genistein and daidzein obtained by using the trapezoidal method (20) was 1.36 ± 0.23, which was the same as the molar ratio of 1.36 for the contents of the isoflavones in the soy flour meal.

Log transformation of the plasma isoflavone concentrations during the period 11–35 h after the meal (study phase 2) suggested a single elimination rate (Figure 4). Mean correlation coefficients for daidzein and genistein were both 0.99 (range: 0.97–1.00). The derived elimination half-lives ($t_{1/2}$), together with the mean maximum concentrations ($C_{max}$) and the mean of the corresponding times to reach maximum concentrations ($t_{max}$), are shown in Table 1. Although both $t_{max}$ and $t_{1/2}$ tended to be higher for genistein than for daidzein, these were not significantly different (Student’s unpaired $t$ test). The higher mean $C_{max}$ value for genistein is consistent with the higher concentration of genistein in the soy meal.

The rates of urinary excretion of daidzein, genistein, and equol during the intervals 0–1, 1–2, 2–4, 4–6, and 6–8 h (phase 1), and 11–12, 12–14, 14–16, 16–18, and 18–35 h (phase 2) after the soy meal are shown in Figure 5. The mean excretion rates for daidzein and genistein increased progressively reaching a peak at 6–12 h after the meal. The rate of excretion of daidzein was greater than that for genistein during all postmeal time intervals. Equol excretion was negligible until after the 6–8-h collection period and then increased steeply. A total urine sample was also collected during the interval 0–11 h after the meal for the phase 2 study, which enabled the calculation of total excretion over the 35-h period after consumption of a soy meal. The mean recoveries of daidzein and genistein were 62 ± 6% and 22 ± 4% of the dose, respectively, which were significantly different ($P < 0.001$, Student’s unpaired $t$ test).

**DISCUSSION**

In the present study we examined in detail the concurrent patterns of plasma isoflavone concentrations and urinary isoflavone excretion after consumption of a single soy meal. In agreement with previous studies (12–16), peak rates of urinary excretion occurred between 6 and 12 h after the meal, with more than one-half of total excretion occurring during the first 12 h. The substantially higher urinary recovery of daidzein compared with genistein, and the large interindividual variations in the fractions of ingested isoflavones excreted in urine, have been uniform observations in other studies (12–17). The interindividual differences may reflect differences in gut microflora populations (13, 17) and it has been concluded (15) that the higher urinary excretion of daidzein reflects a greater bioavailability of this isoflavone. However, in the present study, the ratio of the areas
under the plasma isoflavone concentration versus time curves was the same as the ratio of the concentrations of the two isoflavones in the soy flour meal. In agreement with this, similar plasma daidzein and genistein concentrations were shown previously to be accompanied by large differences in urinary recovery, but plasma concentrations were measured only at 6.5 and 24 h after the meal (13–15) and so areas under the curves could not be calculated. Similarly, consumption of isolated soy protein twice daily for 14 d was also shown to result in a close correspondence between the ratio of daidzein to genistein in the isolate and in plasma obtained at 6.5 h and 7 and 14 d after the meal (21).

Unlike some other flavonoids that may be methylated, there is no evidence that once absorbed the isoflavones undergo any metabolic transformations other than the formation of glucuronide and sulfate conjugates. Thus, it is difficult not to conclude that the bioavailabilities of the two isoflavones were similar as was also concluded by Coward et al (21) in the study referred to above.

The higher urinary excretion of daidzein compared with genistein in urine, there are large variations between the different studies in the reported percentages of the ingested isoflavones excreted. Thus, the mean recoveries of daidzein and genistein in urine in the present study were 62% and 22% of the dose, respectively. These compare well with the data of Lu et al (16), who reported 66.2% and 23.9% excretion for daidzein and genistein, respectively, but are somewhat higher than other values reported (12–15), which range from 16% (13) to 49% (14) for daidzein and from 10% (13, 15) to 16% (14) for genistein. There are several possible explanations for this variation. First, the nature of the soy food may influence bioavailability through differences in the nature of the isoflavone conjugates (25, 26).

Although we did not determine the conjugation pattern in our soy flour, it is likely that it differed from those of the soy milks used in other studies (12–14, 16) because soy milk has a greater proportion of simple glucosides than do less processed foods (25, 26). These differences in conjugation pattern may influence the ease of hydrolysis of the glycosidic bond or bacterial degradation (27, 28) and, hence, bioavailability. Second, in our study, care was taken to ensure that urine samples that were obtained over a period of > 2 h were collected in the presence of preservative (18), or, for samples obtained over shorter collection periods, immediately placed on ice and rapidly subsampled and frozen. Details regarding precautions taken before freezing of samples in other studies (12–16) were not always provided; however, none reported using preservative and it is possible that variations may reflect differences in degradation during preliminary storage. The reason for this large variation between laboratories and within laboratories is important and warrants further study.

Log transformation of the plasma concentrations of the isoflavones over time yielded single slopes, with elimination half-lives for daidzein and genistein of 4.7 and 5.7 h, respectively. These values agree with the values of 2.9–4.4 h for daidzein and 3.8–6.7 h for genistein obtained indirectly by using mathematical derivations from urinary isoflavone excretion rates (12, 16). They are also similar to those obtained for the synthetic isoflavone ipriflavone (11), but shorter than that of daidzein, one of the metabolites of ipriflavone, reported by the same group (11). It is possible that the greater value for the half-life of daidzein obtained by Saito (11) may reflect generation of daidzein from ipriflavone. Interestingly, values for the elimination half-life of genistein similar to those obtained in the present study were reported in rodents (9, 29, 30). However, until information is obtained regarding the extent of biliary excretion of isoflavones in humans, the significance of this similarity is not clear.

It has been generally accepted that dietary flavonoid glycosides must be hydrolyzed to the corresponding less polar agly-
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11–35 h were obtained after consumption of a meal at 2200 after a 6-d washout period during which subjects were asked not to consume soy-containing foods. Note that the data for the time period 0–8 h were obtained after consumption of the meal at 0800 whereas the data for the time period 11–35 h were obtained after consumption of a meal at 2200 after a 6-d washout period during which subjects were asked not to consume soy-containing foods. $\bar{x} \pm$ SEM; $n = 6$.

FIGURE 5. Urinary excretion of daidzein (□), genistein (■), and equol (●) before and at intervals up to 35 h after consumption of a single soy meal that provided 2.8 $\mu$mol daidzein and 3.6 $\mu$mol genistein/kg body wt. Note that the data for the time period 0–8 h were obtained after consumption of the meal at 0800 whereas the data for the time period 11–35 h were obtained after consumption of a meal at 2200 after a 6-d washout period during which subjects were asked not to consume soy-containing foods. $\bar{x} \pm$ SEM; $n = 6$.

Cones before significant gastrointestinal absorption can occur (7, 31, 32). However, recent evidence suggests that some ﬂavonoids may also be absorbed as glycosidic forms (33, 34), although the isoflavones have not been studied speciﬁcally. Several aspects of the present study are relevant to this question. The times required to reach peak plasma concentrations for genistein and daidzein were 8.4 and 7.4 h, respectively, with plasma concentrations remaining above 50% of their maximum values for $\leq 12$–16 h after a single soy ﬂour meal. The times to reach maximum plasma concentrations are much longer than the value of 1.3 h for the synthetic isoflavone aglycone, ipriflavone (11). This suggests the need for hydrolytic cleavage of the glycosidic bond of isoflavone glycosides of soy before signiﬁcant absorption can occur. In the present study isoflavones were detectable in plasma 30 min after the meal, and concentrations at 1 h were $\approx 40\%$ of their peak values. Based on the value of 3% of total isoflavones present as aglycones in our soy meal, $\approx 6$ $\mu$mol daidzein was ingested as the aglycone. The urinary excretion rate for daidzein during the ﬁrst hour showed that 2.5 $\mu$mol was excreted during this period. With use of our value of 62% of ingested daidzein excreted in urine, the daidzein absorbed during the ﬁrst hour could be accounted for by the 3% aglycone present in the soy meal. Similar arguments apply to genistein. For a liquid meal, 50% gastric emptying occurs within 40 min of consumption with a median small intestinal transit time (pylorus to cecum) of 2–3 h (35). Thus, the rapid rise in plasma concentrations seen during the ﬁrst hour may have been due to absorption of aglycone present in the soy meal, with the later absorption occurring after hydrolysis of glycoside conjugates by microorganisms present in the small and large intestines (36). Similar conclusions were drawn in our studies in rats (9). Consistent with this conclusion is the appearance of signiﬁcant concentrations of equol in urine only at times later than 6–8 h after the meal, probably reﬂecting bacterial degradation of the isoflavones after entry of the meal into the large intestine.

As discussed earlier, care should be taken in generalizing the present results. Both bioavailability and the time course of hydrolysis of conjugates, as well as their transport across the gut wall and appearance in blood, may depend on the nature of the soy food involved. It will therefore be important in future comparative studies to determine the inﬂuence of conjugation patterns on isoflavone pharmacokinetics and bioavailability.

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REFERENCES


