Bioavailability, Disposition, and Dose-Response Effects of Soy Isoflavones When Consumed by Healthy Women at Physiologically Typical Dietary Intakes1

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ABSTRACT The pharmacokinetics of isoflavones in 10 healthy women were determined from serum appearance/disappearance concentration profiles and urinary excretions after single-bolus ingestion of 10, 20 or 40 g of soy nuts delivering increasing amounts of the conjugated forms of daidzein (6.6, 13.2 and 26.4 mg) and genistein (9.8, 19.6 and 39.2 mg). Peak serum daidzein and genistein concentrations were attained after 4–8 h, and elimination half-lives were 8.0 and 10.1 h, respectively. There were no differences in the pharmacokinetics of daidzein and genistein between pre- and postmenopausal women, indicating absorption and disposition of isoflavones to be independent of age or menopausal status. A curvilinear relationship was observed between the bioavailability of daidzein and genistein, apparent from the area under the curve to infinity (AUCinf) of the serum concentration-time profiles and the amount of isoflavones ingested. The mean fraction of the isoflavones excreted in urine decreased with increasing intake when expressed as a percentage of the administered dose (63.2 ± 8.0, 54.4 ± 8.1 and 44.0 ± 4.3%, respectively, for daidzein, and correspondingly, 25.2 ± 5.3, 13.4 ± 2.1 and 15.8 ± 2.7% for genistein), underscoring the trend toward nonlinear pharmacokinetics. Equol was identified as a metabolite in 30% of women; it was present consistently in urine and blood from the same subjects. Its delayed appearance was consistent with colonic synthesis. On the basis of the pharmacokinetics, optimum steady-state serum isoflavone concentrations would be expected from modest intakes of soy foods consumed regularly throughout the day rather than from a single highly enriched product. J. Nutr. 133: 1027–1035, 2003.

KEY WORDS: • phytoestrogen • isoflavone • pharmacokinetics • soy foods • humans

There is now a considerable evidence showing potential beneficial effects of dietary phytoestrogens for many hormone-dependent conditions (1–3). Soy foods made from whole soybeans, or isolated soy proteins all contain relatively high concentrations of isoflavones, primarily in the form of various β-glycoside conjugates (4–6). When ingested, these conjugated isoflavones undergo hydrolysis, releasing the principal bioactive aglycones, daidzein and genistein (7–9). These aglycones and any bacterial metabolites, of which equol may prove to be the most important (10,11), are then absorbed from the intestinal tract to undergo enterohepatic recycling (12). These aglycones act as partial estrogen agonists and antagonists; they also exert nonhormonal effects on the cell machinery (13–18) that may be important in explaining some of the health claims and observed physiologic effects of soy protein diets. Intestinal metabolism is therefore essential for the absorption and bioavailability of isoflavones in most foods because there is no evidence to support absorption of the conjugated forms of isoflavones in humans (9).

Following our early hypothesis that phytoestrogens play an important role in hormone-dependent conditions (7), soy isoflavones have been investigated for their cholesterol-lowering actions (19–21) as an alternative to conventional hormone replacement for postmenopausal women, and for their effects on bone (22–24). The results from these studies have been conflicting, which may be more a reflection of differences among the study designs, sources of isoflavones used and levels of intakes. Because there are presently no guidelines for optimal levels of isoflavones and there is a paucity of data on their pharmacokinetics (1,25–27), dietary intakes used in clinical studies have largely been empirically derived. A daily intake of ≥50 mg of isoflavones, which has generally been used in clinical studies, seems to have been based largely on the early observation that daily consumption of soy foods containing 45 mg isoflavones caused endocrine modulation of the menstrual cycle of premenopausal women (28,29).

How varying dietary isoflavone intake influences the bio-
availability and pharmacokinetics is largely unknown. Recent estimates of dietary intakes in people living in Asian countries indicate that intakes of isoflavones from soy foods range from 15 to 50 mg/d (30–32), significantly lower than doses currently being used in clinical studies. In an ongoing program to examine factors that influence isoflavone pharmacokinetics, we have determined how intakes within the usual dietary range typical of people consuming traditional Asian diets influence their bioavailability, pharmacokinetics and metabolism in pre- and postmenopausal women.

SUBJECTS AND METHODS

Study design. Healthy women (n = 10) ≥18 y old were recruited for this study which was carried out utilizing the resources of the NIH-funded General Clinical Research Center at Children’s Hospital Medical Center, Cincinnati, OH. They comprised 5 premenopausal and 5 postmenopausal women, with the latter defined by at least 2 y from the time of last menses. Subjects with preexisting chronic renal, liver, pulmonary or cardiovascular disease, or who had been administered antibiotics within the preceding 3 mo, or were taking oral contraceptives or hormone replacement therapy were excluded. The study protocol was approved by the Human Investigations Review Board of the Children’s Hospital Medical Center and informed consent was obtained from each subject.

Subjects were asked to abstain from foods containing soy protein at least for 1 wk before and during the study. After an overnight fast, each individual was given on separate occasions 10, 20 or 40 g of toasted soy nuts (Country Life Natural Foods, Pullman, MI) to eat in a single bolus. A minimum of 1 min elapsed between consecutive intakes of the different amounts of soy nuts, and the sequence in which each dose was given was randomized to minimize possible carry-over, or period effects. This randomized crossover-washout design served to increase the statistical power of the study. The same batch of soy nuts was used throughout the study and its isoflavone composition was characterized by reversed-phase HPLC (5). The amount of soy nuts consumed was designed to deliver as closely as possible 15, 30 and 60 mg in total of isoflavones expressed as aglycones. These intakes span the usual range of dietary intake in persons living in Asia where soy isoflavones are a staple of the diet (30–32).

Blood samples (5 mL) were obtained by venipuncture, before (baseline) and then 2, 4, 6, 8, 12, 24, 36 and 48 h after consuming the soy nuts, and during each of the periods of the crossover design. A venous indwelling catheter was placed in an antecubital vein for the administration of the 13C-labeled isoflavone. Serum samples were centrifuged at 1200 × g and the serum separated and immediately frozen at −20°C. Isoflavones were measured in the serum by gas chromatography-mass spectrometry (GC-MS) as described previously (27). The blood samples were centrifuged at 1200 × g and the serum separated and immediately frozen at −20°C. Isoflavones were measured in the serum by gas chromatography-mass spectrometry (GC-MS) as described previously (27).

Two 12-h pooled pooled urine collections were obtained before ingestion of the soy food; 12-h urine pools were obtained thereafter for 5 consecutive days. Concentrations of the isoflavones, daidzein and genistein, and the biologically active intestinal bacterial metabolite, equol, were determined in these samples by GC-MS techniques developed in this laboratory (27). To assess oral-anal gastrointestinal transit time, each subject was given a 100-mg capsule of carmine red dye on the initial visit to the clinic and the time of appearance of the first colored stool was recorded by the subject (33).

Analytical methodology

Analysis of soy nuts for isoflavones by electrospray ionization-mass spectrometry (ESI-MS) and HPLC. Soy nuts (5 g) were ground to a very fine powder using a small coffee grinder, and an accurately weighed portion of each was extracted into 80% methanol (50 mL) by refluxing for 1 h. After filtering through a Whatman #1 filter paper (Fisher Scientific, Pittsburgh, PA), the aqueous methanolic phase was made up to a fixed volume of 100 mL in a volumetric flask. An internal standard, equilin (60 g) (Steraloids, Newport, RI), was added to a 1.0 mL (1%) portion of this extract which was then dried, redissolved in the HPLC mobile phase and analyzed directly by HPLC with UV and ESI-MS detection. The conditions employed for reversed-phase HPLC and ESI-MS were reported previously (27). The reproducibility of the method established by interbatch replicate analyses of a quality assurance sample was 2.7% (CV).

Determination of isoflavone pharmacokinetics. The concentrations of daidzein, genistein and equol in serum (0.5 mL) and urine (0.5 mL) were measured by mass spectrometry after the addition of stable isotopically labeled internal standards for quantification. Isoflavones were extracted on a solid-phase octadecylsilane-bonded silica cartridge (Varian, Harbor City, CA), hydrolyzed enzymatically with a mixed β-glucuronidase/sulfatase preparation (Sigma, St Louis, MO); after reextraction and purification, the tert-butyldimethylsilyl (Regis, Morton Grove, IL) ether derivatives were prepared of the serum samples only. These were analyzed by selected ion monitoring GC-MS as described previously (27). The urine samples were analyzed by HPLC with ESI-MS and selected ion monitoring without being derivatized. The hydrolyzed urinary isoflavone extracts were redissolved in 100 μL of the mobile phase. The sample size injected on column was 10 μL, and the mobile phase flow rate was 1.0 mL/min. Separation of the individual isoflavones was accomplished on a 250 × 4.6 mm ODS (C18) reversed-phase HPLC column (Keystone Scientific, Bellefonte, PA). The column was eluted with a water/acetonitrile gradient. The mobile phase was 100% of 10 mmol/L ammonium acetate (1.0 g/L trifluoroacetic acid (TFA)) held isotric for the first 2 min and then decreased to 50% in a constant gradient from 2 to 24 min, and then finally held isocratic with 50% acetonitrile and 50% 10 mmol/L ammonium acetate (1.0 g/L TFA) for a 5-min period before returning to the original composition of 100% 10 mmol/L ammonium acetate (1.0 g/L TFA). ESI-MS was performed on a Micromass Quattro LC/MS. The HPLC effluent to the ESI probe was split 10:1. The desolvation temperature was 300°C, and the source temperature was 100°C. The sampling cone was held at 50 V, and the extractor at 2 V. Data were collected in the positive ion mode and the (M+H)+ ions monitored were m/z 255 (daidzein), m/z 256 ([13C]daidzein), m/z 239 (equol), m/z 240 ([13C]equol), m/z 271 (genistein) and m/z 272 ([13C]genistein). The isoflavone concentrations were established by comparing the isoflavone peak area ratio to that of the corresponding stable-labeled internal standards and interpolating these ratios against calibration plots constructed of known quantities of the pure compounds.

Analysis of soy nuts for isoflavones by electro spray ionization-mass spectrometry (ESI-MS) and HPLC. Soy nuts (5 g) were

RESULTS

Isoflavone composition of the soy food. Analysis of five separate 5-g samples of soy nuts revealed the mean total isoflavone concentration to be 2633 ± 105 μg/g. Soy nuts contained predominantly daidzein and genistein, and these were found mainly in the form of their B-glucoside conjugates.
Only traces of malonyl- and acetyl-glycosides were present and these were too low for reliable quantification. Glycitin was also identified but it accounted for only 2.4% of the total isoflavones in the soy nuts. The aglycones, daidzein and genistein, each accounted for more than 2.5% of the total isoflavones in the soy nuts. The ratio of genistein/daidzein conjugates and aglycones was 1.49 and this varied little (CV = 1.3%) among the different samples of soy nuts analyzed. The different amounts of soy nuts consumed delivered a total of 16.38, 32.76 and 65.52 mg of isoflavones, consisting mainly of genistein and daidzein as various glycosidic conjugates. On the basis of these analyses, the study subjects ingested a total of 6.59, 13.18 and 26.36 mg equivalents of daidzein with the three increasing doses of soy nuts consumed. Correspondingly, the amounts of genistein ingested were 9.79, 19.58 and 39.16 mg, respectively.

Demographics of the study population. The age, body weight, and the body mass index (BMI) of the study subjects are detailed in Table 1. The age of the pre- and postmenopausal women differed (P = 0.027) as expected. The BMI of the premenopausal women tended to be higher (P = 0.14) than that of the postmenopausal women.

Serum kinetics of isoflavones. The serum appearance/disappearance curves established that daidzein and genistein were absorbed soon after ingestion. The maximum serum concentration was attained on average at 6.9 ± 0.7 h for daidzein and 6.5 ± 1.0 h for genistein after ingestion of soy nuts, indicating that absorption of isoflavones occurs along the length of the small and large intestine, with little absorption taking place in the stomach. As previously shown for pure compounds (27), there was a consistent early kink during the appearance phase of the serum isoflavone concentration profiles in all subjects (not as evident when the data were grouped and averaged), and this is consistent with compounds undergoing enterohepatic recycling. Figure 1 shows the mean serum concentrations of daidzein and genistein plotted on a log/linear scale for 9 of the 10 study subjects after ingesting increasing levels of isoflavones from soy nuts. Excluded from the group are data from one of the premenopausal women (Table 1, Subject #3) who exhibited a consistently prolonged terminal elimination phase and delayed time to achieve peak levels at all three dietary intakes of isoflavones. The computed pharmacokinetics for daidzein and genistein in this subject yielded values that were >2 SD from the means of the rest of the group. Notable were the computed serum t1/2 of 18.5 ± 0.6 h for daidzein and 30.7 ± 7.7 h for genistein. The reasons for this delayed elimination are unknown but the consistency with which this was observed suggested that this subject was unique in her handling of isoflavones. For this reason, her data were excluded from the group analysis.

Table 2 summarizes the data obtained when these serum values were computed using the noncompartmental pharmacokinetic approach for the bioavailability as measured from the area under the curve to infinity (AUCinf), the t1/2, the CI/F and the Vd/F for the nine subjects. Pharmacokinetics were calculated with and without exclusion of outlying data points with negligible differences observed between the two approaches. Overall, the t1/2, CI/F and Vd/F for the nine subjects were similar and independent of the levels of intake. For daidzein, the mean half-life was 8.7, 7.9 and 7.5 h with the three increasing dietary intakes of isoflavones, respectively, and correspondingly, the values for genistein were 11.1, 10.0 and 9.6 h. Because half-life is independent of dose administered, combining all data established a group mean (±SEM) half-life for daidzein of 8.0 ± 0.3 h, whereas for genistein, this value was 10.1 ± 0.3 h.

When these data were subgrouped according to menopausal status, utilizing a t test (two-tailed, type 2), no significant differences in the AUCinf of daidzein (P = 0.71, 0.45 and 0.51) or genistein (P = 0.98, 0.56 and 0.68) were observed between pre- and postmenopausal women at the respective doses of intake. Similarly, no significant differences in the t1/2, CI/F and Vd/F between pre- and postmenopausal women were observed. Within the constraints of the small sample size, this would suggest that the absorption and disposition of daidzein and genistein are similar in adult women irrespective of their age or menopausal status (Table 2). Application of post-hoc analysis to AUC values of the pre- and postmenopausal groups indicated that to observe a significant difference in AUC in this experiment, a minimum of 336 subjects per group would have been necessary for 80% power. This is based on a two-tailed t test at the α = 0.05 level. The most striking observation, relative to the dietary isoflavone intake, was the lack of

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**TABLE 1**

Demographics of five premenopausal women and five postmenopausal women ingesting increasing amounts of soy nuts

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Body weight</th>
<th>Body mass index</th>
<th>Gut transit time at each visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>kg</td>
<td>kg/m²</td>
<td>h</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44</td>
<td>72.6</td>
<td>30.1</td>
<td>10.1</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>91.5</td>
<td>38.0</td>
<td>7.1</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>81.6</td>
<td>34.0</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>64.0</td>
<td>26.6</td>
<td>8.7</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>61.1</td>
<td>25.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>39 ± 3.6</td>
<td>74.0 ± 5.6</td>
<td>30.8 ± 2.6</td>
<td></td>
</tr>
</tbody>
</table>

Post-menopausal |     |             |                 |                               |
| 6       | 53  | 53.6        | 22.3            | 10.1                          |
| 7       | 49  | 54.0        | 22.4            | 10.1                          |
| 8       | 56  | 62.2        | 25.8            | 10.1                          |
| 9       | 56  | 68.8        | 28.6            | 10.1                          |
| 10      | 56  | 75.1        | 31.2            | 10.1                          |
| Mean ± SEM | 54 ± 1.4 | 63 ± 4.2 | 26.1 ± 1.9 |

1 * indicates the dye was not visualized by the subject.
2 This subject was not included in plasma analysis because at all three dietary intakes of isoflavones she exhibited a consistently prolonged terminal elimination phase and delayed tmax.
linearity in the bioavailability as apparent from the AUC\textsubscript{inf} of the serum concentration-time profiles. Rather than a doubling of the AUC\textsubscript{inf} with a doubling of the isoflavone intake, a curvilinear relationship was obtained. Figure 2 depicts the plots for AUC\textsubscript{inf} vs. daidzein and genistein intake. The line of linearity has been included for comparing the observed AUC vs. dose curve against the plot that would be observed if the AUC increased linearly with dose. The magnitude of deviation from linearity was greater for genistein than for daidzein. Another approach to demonstrate the nonlinearity is to plot dose-normalized AUC\textsubscript{inf} vs. the dose of each isoflavone ingested (Fig. 2). Usually, within the dose-range in which the pharmacokinetics are linear, the AUC/dose ratios should be more or less constant. The fact that these consistently decreased for both isoflavones indicates nonlinear pharmacokinetics.

It is important to point out that when evaluating the observed AUC\textsubscript{inf} vs. dose relationship on an individual basis, a nonlinear decrease in the AUC\textsubscript{inf} was not observed for daidzein and genistein in 3 of 10 subjects. The reasons for these apparent “outliers” are not clear. One possibility is that we employed only three doses, and variability in the AUC\textsubscript{inf} at one or more dose level would therefore have a large effect on the overall trend. Another important point to note is that the t\textsubscript{1/2}, CL/F and Vd/F of daidzein and genistein did not change significantly (P > 0.05) with change in dose.

Orol-anal transit times of carmine red dye that was given to each subject at each of the three visits are presented in Table 1. There was some reported difficulty in visualizing carmine red in the stools, with the subjects recording a “time of observation” in only 13 of the possible 30 visits (10 subjects × 3 levels of soy nuts). The oral-anal transit time of carmine red was highly variable among those subjects that could visualize the dye, and ranged from 35 to 120 h. Conclusions regarding the effect of intestinal transit time on the pharmacokinetics were therefore difficult to make with confidence but there was little evidence for any correlation between the recorded transit times and any of the serum pharmacokinetic measures.

**Urinary isoflavone excretion.** The daily excretion of daidzein and genistein in urine at baseline and over 5 consecutive days after the ingestion of 10, 20 and 40 g of soy nuts is depicted in Figure 3. Most of the excreted isoflavone was eliminated within the first 2 d, although there continued to be elimination of small proportions up to 5 d later, as evidenced from the continued rise in the cumulative excretion curves (Fig 4). This is consistent with the observed t\textsubscript{1/2} of 8–10 h of the two isoflavones, which would predict that ~95% of the

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Soy nuts, 10 g bolus</th>
<th>Soy nuts, 20 g bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>Daidzein</td>
<td>Daidzein</td>
</tr>
<tr>
<td>Prenopausal</td>
<td>407 ± 103</td>
<td>583 ± 120</td>
</tr>
<tr>
<td>Post-prenopausal</td>
<td>446 ± 137</td>
<td>1,050 ± 320</td>
</tr>
<tr>
<td>All Women</td>
<td>428 ± 79</td>
<td>843 ± 188</td>
</tr>
<tr>
<td>Genistein</td>
<td>Genistein</td>
<td>Genistein</td>
</tr>
<tr>
<td>Prenopausal</td>
<td>679 ± 206</td>
<td>1,055 ± 345</td>
</tr>
<tr>
<td>Post-prenopausal</td>
<td>511 ± 139</td>
<td>1,446 ± 472</td>
</tr>
<tr>
<td>All Women</td>
<td>585 ± 109</td>
<td>1,222 ± 297</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 4 premenopausal and 5 postmenopausal women.

2 $C_{\text{max}}$ = peak serum concentration.

3 $t_{\text{max}}$ = time required to achieve peak levels.

4 $t_{1/2}$ = serum elimination half-life.

5 Vd/F = volume of distribution normalized to the bioavailable fractional.

6 CL/F = systemic clearance normalized to bioavailable fractional.

7 AUC\textsubscript{inf} = bioavailability as apparent from area under the curve to infinity.
Isoflavones would be eliminated within the first 2 d after ingestion, with very small amounts excreted thereafter.

The fractional absorption of daidzein and genistein as measured by the amounts excreted in urine relative to the amount ingested on a body weight basis was relatively constant, but there was a trend toward decreased fractional absorption at the higher level of intake (Fig. 5). This was not significant due to the large interindividual variation in urinary excretion. The mean fraction of daidzein excreted in urine (expressed as a percentage of the administered dose) was 63.2 ± 8.0, 54.4 ± 8.1 and 44.0 ± 4.3% when 10, 20 and 40 g of soy nuts were ingested, respectively. The corresponding values for genistein were much lower at 25.2 ± 5.3, 13.4 ± 2.1 and 15.8 ± 2.7%, respectively. These values are consistent with the observed nonlinear increase in the AUC_{inf} with increased doses. The fraction of a xenobiotic dose excreted in urine is directly proportional to the systemically bioavailable fraction. When a compound exhibits linear pharmacokinetics over a dose range, this fraction remains unaltered. However, for both daidzein and genistein, there was a decline in the fraction excreted in urine from the lowest to the highest level ingested, underscoring the trend toward nonlinear pharmacokinetics. A consistent observation we have made is the suggestion of a second increase in daidzein and genistein levels as late as d 4–5, which was more evident when individual profiles are examined, but the reason for this is unclear.

The relatively poor recovery of daidzein and genistein has been a consistent observation in all studies and is possibly explained by extensive metabolic degradation of the isoflavones by intestinal bacteria to as yet undefined metabolites.
the known metabolites of soy isoflavones, we measured only equol because at the time of these studies, this was the only reference standard available for quantification. Other metabolites have been reported to constitute only minor concentrations in urine. Serum and urinary equol was identified in only 3 of the 10 subjects studied. The mean cumulative excretion of equol for these three subjects ingesting 10, 20, and 40 g of soy nuts is shown in Figure 6. In all cases, the same subjects produced equol on all three occasions they were challenged with differing amounts of soy nuts. As is evident from the cumulative excretion data, it took some time before equol appeared in the urine, and maximum levels were not observed until the later time points, consistent with its colonic origin. Interestingly, equol proved difficult to quantify in serum samples due to its presence at levels close to the methodological detection limit.

**DISCUSSION**

It has been known for many decades that soybeans and most soy foods contain isoflavones in relatively high concentrations; genistin was first isolated from soybeans by Walz in 1931, and later by Walter in 1941. The dietary intake of this class of phytoestrogens can be substantial in people consuming soy foods on a regular basis, as was suggested. This class of phytoestrogens can be substantial in people consuming soy foods on a regular basis, as was indicated that soy protein contains isoflavones, we measured only equol because at the time of these studies, this was the only reference standard available for quantification. Other metabolites have been reported to constitute only minor concentrations in urine. Serum and urinary equol was identified in only 3 of the 10 subjects studied. The mean cumulative excretion of equol for these three subjects ingesting 10, 20, and 40 g of soy nuts is shown in Figure 6. In all cases, the same subjects produced equol on all three occasions they were challenged with differing amounts of soy nuts. As is evident from the cumulative excretion data, it took some time before equol appeared in the urine, and maximum levels were not observed until the later time points, consistent with its colonic origin. Interestingly, equol proved difficult to quantify in serum samples due to its presence at levels close to the methodological detection limit.

It was difficult to ignore the level of enthusiasm for isoflavones as potential factors in either preventing or treating a range of diseases, and this is evident from the large numbers of dietary intervention studies that have been performed to date. However, there has been a tendency in clinical trials to use relatively large dietary intakes of soy isoflavones derived from soy beverages, foods, or supplements, far exceeding typical intakes for people living in Asian countries (30–32); the rationale for this is unclear and not founded on any knowledge of their pharmacokinetics. As with pharmacological agents, demonstrating efficacy of soy isoflavones is contingent on their bioavailability and in this regard, there is little information of how this varies among individuals, whether it is influenced by age, or what the effect of ingesting levels that far exceed normal dietary intakes has on their pharmacokinetic behavior. The influence of the food matrix remains to be elucidated.

To our knowledge, there are few studies of the serum pharmacokinetics of daidzein or genistein (25–27,41–43) and certainly none that has involved such extensive serum sampling as we report here. Our data represent the most comprehensive information on the pharmacokinetics of daidzein and genistein when these isoflavones are ingested in their natural forms from a soy food, in this case soy nuts. The studies were performed according to a classical oral single-bolus pharmacokinetic design and complement our earlier reports of the pharmacokinetics of the pure compounds, thereby permitting historical comparisons of these data. The magnitude of the study, with >600 samples having been measured by MS, precluded effects of chronic exposure to soy isoflavones to be addressed separately. Our recent studies using [13C]stable-labeled analogs of daidzein and genistein, however, found the pharmacokinetics of these two isoflavones in healthy premenopausal women to be unaffected by a background of persistent isoflavone ingestion from soy foods (44). The intakes of between 6.59 and 26.36 mg daidzein and 9.79 and 39.16 mg genistein (expressed as the aglycone equivalents) reflect the usual dietary intakes of people consuming soy as a staple food (30–32). These levels correspond to doses of up to ~0.4 mg/kg body for daidzein and 0.75 mg/kg body for genistein.

Daidzein and genistein were absorbed relatively quickly, attaining maximum serum concentrations at times ranging from 2 to 8 h after ingestion among the subjects, with means for daidzein and genistein of 6.1 and 5.0 h, respectively. The bioavailability, as apparent from the AUCint, showed a curvilinear relationship with increasing levels of isoflavones ingested. This curvilinear relationship becomes more evident at even higher isoflavone intakes as we observed recently in studies of [13C]daidzein and [13C]genistein given in the dose range 0.4–1.8 mg/kg body (44). Taken together, the serum and urinary data confirm that the pharmacokinetics of daidzein and genistein are nonlinear. Usually, when a compound exhibits nonlinear pharmacokinetics, the AUCint increases in a manner that is disproportionate to the applied dose. When the increase in AUCint is higher than that predicted on the basis of a strictly linear relationship with dose, it usually indicates that the one or more elimination pathways, such as metabolizing enzymes or transporters (renal or biliary), are saturated. When the AUCint increase is less than that expected on the basis of a linear relationship, it is indicative of either increased elimination, which is usually due to induction of metabolizing enzymes, or reduced absorption. In the case of both daidzein and genistein, our observations suggest that the latter mechanism is more likely to account for the observed nonlinearity. Because the t1/2, CI/F and Vd/F were relatively unchanged from one dose to the other, it is unlikely that there were any dose-dependent changes in the clearance pathways. Therefore, the reduced systemic availability is most likely explained by...
reduced absorption of isoflavones with increasing levels of intake.

The mean t1/2 of daidzein and genistein was 8.0 ± 0.3 and 10.1 ± 0.3 h, respectively, and, as expected, independent of the amount ingested. These values are similar to those of the pure compounds when administered orally (27), and of the [13C]labeled tracers (44). They are, however, longer than previously reported values for isoflavones in other soy foods (26,41) given to healthy adults. This difference may be explained by the effect of certain food matrices on the dynamics of absorption and elimination. Significantly longer half-lives are found in patients with renal disease, i.e., times of up to 53 and 99 h for genistein and daidzein, respectively (42), and this is because renal clearance is the major route for their elimination from the body. One of the women enrolled had explained excessively prolonged t1/2 for daidzein and genistein and 99 h for genistein and daidzein, respectively (42), and this is explained by the effect of certain food matrices on the dynamics of absorption and elimination. This difference may be explained by the effect of certain food matrices on the dynamics of absorption and elimination. This difference may be explained by the effect of certain food matrices on the dynamics of absorption and elimination.

Daidzein is always excreted in greater amounts than genistein in the urine of adults when soy foods are consumed (46–51) (52–54). It is relevant to mention that some investigators have incorrectly assumed that this implies that daidzein is more bioavailable than genistein (25,55). It should be pointed out that the bioavailability of any drug or compound can be measured only from its urinary excretion if it is 100% bioavailable and recovered completely in the urine. As is evident from many studies, this is not the case for soy isoflavones for which the fractional recovery of daidzein and genistein in urine is rather low (Fig. 5). Accurate measurement of bioavailability requires comparing the AUCinf after oral and intravenous administration of the pure compounds. On a practical note, the accuracy of this determination is critically dependent on obtaining multiple blood samples during the elimination phase and sampling times should ideally be extended to at least five half-lives beyond the time of the steady-state serum concentration. Only a few studies on bioavailability have taken this into consideration (26,27,41,42,44) and in some, the kinetics were computed using only two time points (25,43), where the later measurement was close to the limits of detection of the method and subject to poor precision. These factors undoubtedly account for the variability and discrepancies in the literature.

The apparent systemic exposure to genistein as computed from the AUCinf was greater than that of daidzein (this measure takes into account the fact that there were different levels of intake in the soy nuts and is independent of the composition of the food). This has been a consistent finding from studies also using the individual pure compounds (27) and the stable isotopically labeled tracers (44). It also explains why serum genistein concentrations are consistently higher than those of daidzein when most soy foods are fed (26,41,43). Other pharmacokinetic studies have suggested that there are no differences in bioavailability of daidzein and genistein in healthy subjects (26), or that daidzein is more bioavailable than genistein (25). Altered pharmacokinetics are observed in patients with impaired kidney function, where serum daidzein far exceeds genistein concentration (42).

We found no significant age-related differences in any of the means of the lambda z, CI/F, Vd/F, t1/2, and AUCinf between pre- and postmenopausal women. Notwithstanding the small sample size, these findings indicate that the fate of isoflavones is not influenced by a woman’s age or menopausal status, and that their bioavailability is similar, at least from a food such as soy nuts.

One of the consistent findings in this and other studies is the inability to account for all of the isoflavone ingested. The fractional recoveries in urine of daidzein and genistein were highly variable among individuals, ranging from 18 to 95% (mean 50%) for daidzein and from only 5 to 42% (mean 16%) for genistein. Similar low recoveries and variations have been noted in other studies of urinary isoflavone excretion. This suggests that there are marked individual differences in the intestinal metabolism of isoflavones and that there are likely some metabolites yet to be identified and measured. Daidzein and genistein are formed from hydrolysis of the β-anglensin, dihydrogenistein, equol, p-ethylphenolic acid and p-ethylphenol. To our knowledge, with the exception of equol, these metabolites are hormonally inactive (11). At the time of initiating our studies, we did not have pure standards to quantify all of these metabolites. However, other investigators have shown that these metabolites are present in urine at only a fraction of the levels of daidzein and genistein. Equol may prove to be the most important isoflavone metabolite (11) because it has a greater affinity for binding to the estrogen receptor than its precursor daidzein (56). Several recent clinical studies (Howe et al., personal communication, P.R.C. Howe, University of Woolagong, NSW, Australia) have found that equol producers, compared with nonequol producers, show greater clinical benefits where hormone-sensitive end points are concerned (24,57) when consuming soy foods. As we first showed, not everyone is capable of producing equol (7) and consistent with this observation, we found equol in the urine and serum of only 3 of the 10 women studied here. Similar observations have been made by others (58,59). Interestingly, these same women produced equol on all three occasions when given different doses of soy nuts, whereas the other women remained nonproducers throughout. This suggests that under normal circumstances, “once an equol producer, always an equol producer.” The formation of equol takes place mainly in the colon and this explains the delay in appearance of equol in the urine. Cumulative excretion curves reveal that equol is continually being produced at the later time points.

What do these pharmacokinetic studies reveal? Our data support the concept that the bioavailability of isoflavones from a soy food matrix such as soy nuts is nonlinear, with increased doses of intake in the range typically consumed by people living in Asia. Because the serum t1/2 of daidzein and genistein are on the order of 8–10 h, steady-state serum levels are more likely to be achieved by multiple intakes of soy foods throughout the day. Whether this would result in increased clinical efficacy in dietary intervention studies is not known, but we contend on the basis of analogy to many drugs with similar pharmacokinetics, that a portfolio of soy foods that can be eaten throughout the day is likely to be more effective than once a day intake. Finally, the advantage of ingesting large doses of soy isoflavones in soy foods is questionable, given the curvilinear relationship between bioavailability and intake. Why food and nutraceutical companies persist in flooding the market with products containing high levels of soy isoflavones...
is unclear when these amounts are far in excess of usual dietary intakes of Asians (30–32) and there exists the potential for long-term negative effects.

ACKNOWLEDGMENTS

We thank Chandra Childress and Pinky Jha for their technical support in these studies.

LITERATURE CITED


