Pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein in men and women after consumption of a soy beverage1–3

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ABSTRACT

Background: The soy isoflavones genistein and daidzein are found in blood and tissues as aglycones, glucuronides, and sulfates. Isoflavone conjugates may serve as sources of aglycones at specific target tissues and may have bioactivity. Yet, very little is known about the plasma pharmacokinetics of isoflavone conjugates after soy ingestion.

Objective: The objective of this study was to determine the plasma pharmacokinetics of glucuronide and sulfate conjugates of genistein and daidzein in humans after the consumption of a drink made with soy-protein isolate.

Design: Six men and 6 women (3 ± SD age: 40.8 ± 3 y) consumed a soy-protein-isolate drink. The pharmacokinetics of isoflavone glucuronide and sulfate conjugates were studied with the use of β-glucuronidase (EC 3.2.1.31) and sulfatase (EC 3.1.6.1) digestion and liquid chromatography–mass spectrometry.

Results: Glucuronides of genistein and daidzein made up a significantly lower percentage (P < 0.05) of the total isoflavone concentration in plasma (48% and 33%, respectively) than in urine. The percentages of sulfates of genistein and daidzein in plasma (8% and 26%, respectively) were 2- to 6-fold those in urine (P < 0.05). Approximately 30% of the total genistein or daidzein was comprised of mixed conjugates (one glucuronide and one sulfate). For daidzein sulfate, genistein sulfate, daidzein glucuronide, and genistein glucuronide, the time to peak (t,,max) was 4.5, 4.5, 4.5, and 6.0 h, respectively, and the apparent half-life (t,1/2,α) was 3.1, 5.7, 3.2, and 8.4 h, respectively.

Conclusions: These data suggest that there are significant differences in the pharmacokinetics of sulfate and glucuronide conjugates of isoflavones. This may have important implications for the meal frequency and maintenance of target tissue bioactivity required to elicit potential health benefits.

INTRODUCTION

The potential health benefits of dietary soy reportedly range from reduction of plasma cholesterol concentrations to reduction of breast cancer incidence (1). Associated with soy protein are several phytochemicals, including isoflavones, which have been reported to modulate a wide variety of cellular processes and physiologic events responsible for these health effects (for review see reference 2). Genistein and daidzein are the 2 most widely studied soy isoflavones in human diets, and the most commonly reported effects of these aglycones involve estrogenic actions (3).

Most soy infant formulas contain soy-protein isolate (SPI) as the sole source of protein, and SPI is an excellent source of isoflavones. The potential adverse effects of early estrogenic exposure have given rise to safety concerns about feeding soy-based infant formula (4, 5), primarily because the isoflavones attain high circulating concentrations in infants (6). Similar concerns about soy isoflavones have arisen in adults, especially in relation to breast cancer risk in women with occult tumors (for review see reference 7).

Soy isoflavones circulate in several molecular forms, including glucuronide and sulfate conjugates, freely circulating aglycones, and protein-bound aglycones (2, 3, 8, 9). Most absorbed isoflavones are excreted as conjugates into the urine, but a smaller percentage undergo enterohepatic recycling (3, 7). There are 2 conjugation sites on genistein and on daidzein, and each of these sites can be sulfated or glucuronidated. Thus, there are monoglucuronides, monosulfates, diglucuronides, disulfates, and mixed conjugates with one site glucuronidated and one site sulfated (Figure 1). Without sophisticated equipment such as liquid chromatography–tandem mass spectrometry (LC-MS-MS), the lack of commercially available standards for conjugates has been a hindrance in their qualitative and quantitative determination, and this has led to the use of enzymatic digestion of the isoflavone conjugates with subsequent detection of the aglycones (10–12).

Currently, there are 3 commercially available enzyme preparations that are primarily used by most investigators to study conjugates of genistein and daidzein: a Helix pomatia
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preparation, a highly enriched β-glucuronidase (EC 3.2.1.31), and an enriched sulfatase (EC 3.1.6.1). Typically, the conjugated isoflavones in samples of urine or plasma are hydrolyzed with these enzymes, the liberated aglycones are separated and quantitated by HPLC or LC-MS, and the isoflavone concentrations are expressed as aglycone equivalents. The H. pomatia preparation contains glucuronidase and sulfatase activities toward the conjugates of genistein and daidzein in sufficient ratios to determine the total aglycone concentrations of plasma, urine, and tissues. The β-glucuronidase and sulfatase preparations are used to determine the glucuronide and sulfate concentrations, respectively.

To assess the potential risks and benefits of soy isoflavones in infants and adults and the mechanisms by which health effects occur, it is essential to have a more complete understanding of isoflavone pharmacokinetics after consumption of soy foods. Most previous investigators have focused on the pharmacokinetics of total isoflavones. There are very few data on the differences or similarities between urine and plasma metabolites and virtually no data on the plasma pharmacokinetics of the major isoflavone conjugates. In the present study, we used the 3 enzyme preparations described above and an inhibitor of β-glucuronidase (d-saccharic,1-4 lactone) to study the plasma pharmacokinetics of genistein and daidzein conjugates of healthy human subjects who consumed a beverage containing SPI. As far as we are aware, this is the first report to determine the plasma pharmacokinetics of the 2 major conjugates of genistein and daidzein in humans after consumption of SPI.

SUBJECTS AND METHODS

Subjects

Study 1 was approved by the institutional Human Research Advisory Committee of the University of Arkansas for Medical Sciences, and all 12 subjects (6 men and 6 women) gave their written consent. The men and the women ranged in age from 27 to 52 y and from 35 to 47 y, respectively. None of the subjects were taking oral contraceptives at the time of the study, were pregnant, or had taken antibiotics in the past 3 mo. All were considered to be generally healthy, and the mean (±SE) weight of the men and the women was 86.0 ± 6.4 and 60.2 ± 0.8 kg, respectively. The urinary excretion rates of these subjects were reported in a previous publication (10).

Most of the subjects were employees of the Arkansas Children’s Hospital Research Institute. The subjects were given a list of phytoestrogen-containing foods and were asked to avoid consuming them for 1 wk before the study. They were also asked to record all foods and drinks ingested during this 1-wk period. This dietary intake information, combined with the analyses of a 24-h urine sample collected the day before the study started and of a baseline blood sample collected the morning of the study, was used to help verify compliance.

On the first day of the study, the subjects fasted overnight and then consumed a soy beverage that had been prepared to provide a dose of 1.0 mg genistein (aglycone) equivalents/kg body wt and 0.6 mg daidzein (aglycone) equivalents/kg body wt. Doses were calculated on the basis of the concentrations of isoflavones in the soy protein as determined by the manufacturer, Protein Technologies International (St Louis). Each gram of soy protein contained 0.72 mg genistein equivalents, 0.39 mg daidzein equivalents, and 0.07 mg glycitein equivalents. The soy beverage was prepared with soy protein and a banana and was diluted with equal parts of pineapple juice and orange juice (except for one subject who requested no banana and another subject who consumed the SPI dissolved in orange juice only). The amounts of genistein, daidzein, and glycitein in bananas, pineapple juice, and orange juice were assumed to be negligible.

Subjects were presented with a nutritious, balanced meal program (formulated by our dietitians to avoid isoflavone-containing foods) and were allowed ad libitum access to these foods for the duration of the study. Blood samples were collected in heparinized tubes at 0.17, 0.33, 0.5, 1.5, 3, 6, 9, 12, 16, 20, 24, 28, 32, and 48 h after ingestion. Samples were centrifuged at 1000 × g for 2 h at 5°C, plasma was removed, and aliquots were stored at −70°C. All urine produced after soy ingestion was collected at 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, and 48 h after ingestion in containers with ascorbic acid and sodium azide (0.1% wt:vol for each) added as preservatives. Samples were stored at −20°C before analysis.

Study 2 was conducted on plasma and urine specimens from 1 man (aged 29 y) and 4 women (aged 36–49 y) who reported consuming soy products daily. Study 2 was also approved by the institutional Human Research Advisory Committee of the University of Arkansas for Medical Sciences, and all subjects gave their written consent. None of the subjects were taking oral contraceptives.
at the time of the study, were pregnant, or had taken antibiotics in the past 4 mo, and all were considered to be generally healthy. The subjects were asked to empty their bladder and then consume the same soy beverage as described above. Urine was collected for the next 24 h, and a unit of blood was collected by the blood bank at the University of Arkansas for Medical Sciences 4 h after consumption of the soy beverage.

Materials

Genistein (5,7,4′-trihydroxyisoflavone) and daidzein (7,4′-dihydroxyisoflavone) were purchased from Indofine Chemical Co, Inc (Belle Mead, NJ). The following were purchased from Sigma Chemical Co (St Louis): type B-1 β-glucuronidase from bovine liver with <3% sulfatase activity, sulfatase type V (aryl-sulfate sulfohydrolase) from H. pomatia with reported sulfatase activity of 15–40 U/mg and glucuronidase activity of 400–600 U/mg (referred to as “sulfatase-glucuronidase” in this paper), sulfatase type VIII from abalone entrails with reported sulfatase activity of 20–40 U/mg and glucuronidase activity <3 U/mg, and d-saccharic, 1-4 lactone (a β-glucuronidase inhibitor).

Enzymatic digestions

Individual plasma samples for study 1 were analyzed for the genistein and daidzein content of the glucuronide conjugates. Plasma pools were constructed by combining equal volumes of plasma from all 12 subjects at each time point, and these were analyzed in triplicate for the glucuronide and sulfate conjugates of genistein and daidzein. Plasma samples (0.5 mL) were digested with sulfatase (100 U), β-glucuronidase (1000 U), or sulfatase-glucuronidase (100 and 1000 U, respectively) at 37°C for 3 h. All samples were extracted twice with 5 mL diethyl ether, and the organic layers were evaporated to dryness at 55°C under nitrogen. Dried extracts were reconstituted in 0.5 mL of a solvent containing a known amount of biochanin A, and the reconstituted extracts were injected into the LC-MS system under conditions reported previously (10) to determine the aglycone concentrations. All results were expressed as nmol/L after normalization with biochanin A.

In study 2, individual plasma (0.5 mL) and urine samples (1 mL) were first incubated with β-glucuronidase (1000 U) for 3 h at 37°C in the presence or absence of the β-glucuronidase inhibitor d-saccharic,1-4 lactone (100 mmol/L). The samples were then split into 2 equal portions. One aliquot was stored at −20°C until extraction and LC-MS analysis (see above), and the other aliquot was further digested with sulfatase (100 U) for 3 h at 37°C in the presence or absence of d-saccharic,1-4 lactone (100 mmol/L) and stored at −20°C until extraction and LC-MS analysis as described above. The plasma and urine samples were also digested with the sulfatase-glucuronidase (100 and 1000 U, respectively) preparation at 37°C for 3 h and analyzed as described above to obtain the total genistein and daidzein concentrations.

Statistical analysis

Noncompartmental pharmacokinetic analysis of data was conducted with the use of WINNONLIN (Pharsight, Mountain View, CA). Data were visually selected for the terminal slope calculation, and linear regression was conducted by using uniform weighting. A best-fit line was calculated after assessment of the residuals and visual inspection of the line. Data are presented as means ± SEMs. Statistical analysis was conducted with the use of SIGMASTAT version 2.0 (Jandel Scientific, San Rafael, CA), and P < 0.05 was considered statistically significant.

RESULTS

A representative profile of the isoflavone separations obtained by LC-MS is shown in Figure 2. The plasma genistein and daidzein glucuronide concentrations over time in 4 representative subjects are shown in Figure 3. There were clear differences in the time course for these 2 isoflavones: daidzein had a later mean time to maximal concentration (t max) but a faster disappearance rate than did genistein. The t max, the peak plasma concentration

![FIGURE 2. A representative liquid chromatography–mass spectrometry chromatograph of isoflavone standards. TIC, total ion chromatograph; SIM, selective ion monitoring; DHD, dihydrodaidzein; DHG, dihydrogenistein; O-DMA, O-desmethylangolensin; IS, internal standard.](image-url)
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The terminal half-life ($t_{1/2z}$), the maximal concentration ($C_{max}$), and the area under the plasma concentration time curve (AUC) of daidzein and genistein glucuronides in the 12 subjects are shown in Table 1. The genistein glucuronide $t_{max}$, $C_{max}$, $t_{1/2z}$, and AUC values for the subjects ranged between 3.0 and 6.0 h ($\bar{x}$: 4.4 h), 435 and 947 nmol/L ($\bar{x}$: 654 nmol/L), 3.6 and 12.3 h ($\bar{x}$: 7.90 h), and 4617 and 13 726 nmol · h/L ($\bar{x}$: 8682 nmol · h/L), respectively. The daidzein glucuronide $t_{max}$, $C_{max}$, $t_{1/2z}$, and AUC values for the subjects ranged between 3.0 and 9.0 h ($\bar{x}$: 5.50 h), 58 and 401 nmol/L ($\bar{x}$: 298 nmol/L), 2.1 and 5.2 h ($\bar{x}$: 3.4 h), and 449 and 3230 nmol · h/L ($\bar{x}$: 2287 nmol · h/L), respectively. No significant differences in pharmacokinetics were observed between the men and the women.

Because the sample volume was in short supply and the sulfate conjugates in the plasma were less concentrated than the glucuronide conjugates, it was necessary to analyze a sample pool to determine the pharmacokinetics of the genistein and daidzein sulfate conjugates. To verify that the analysis of the plasma pool would be representative of the data derived from individual samples, we compared the glucuronide concentrations of the plasma pool with the calculated mean glucuronide concentration from the individual plasma samples for each time point (Figure 4). As expected, when plotted in the same graph, the time course for the calculated mean was nearly superimposed on that from the plasma pool, providing confidence that the pharmacokinetic data on sulfate conjugates derived from the pool would be a good approximation of those derived from individual samples.

A plasma pool was made by combining equal volumes of plasma from all subjects. The time course for genistein and daidzein sulfate in the plasma pool is shown in Figure 5, and the corresponding pharmacokinetic data are shown in Table 2. For plasma daidzein sulfate, $t_{max}$ was 4.5 h and $t_{1/2z}$ was 3.1 h. For plasma genistein sulfate, $t_{max}$ was 4.5 h and $t_{1/2z}$ was 5.7 h. The AUCs for daidzein and genistein sulfates did not differ significantly. For comparison, the pharmacokinetics of daidzein and genistein glucuronides are also shown in Table 2. The pharmacokinetics of daidzein sulfate and daidzein glucuronide were not significantly different: both had a $t_{max}$ of 4.5 h and $t_{1/2z}$ of 3.1–3.2 h. The only major pharmacokinetic difference between the daidzein and genistein conjugates was the greater plasma concentrations for genistein. Genistein sulfate conjugates had shorter $t_{max}$ and $t_{1/2z}$ values and lower $C_{max}$ and AUC values than did daidzein sulfates. Note that the concentration of circulating free genistein or daidzein was < 1 nmol/L in these samples and was thus considered negligible for calculation purposes.

Preliminary data from our laboratory indicated that the total plasma or urine yields of genistein or daidzein after digestion with the sulfatase-glucuronidase preparation did not equal the yields of these isoflavones derived from digestion with β-glucuronidase plus sulfatase. In study 2, the plasma or urine samples from subjects who drank a soy beverage were digested with β-glucuronidase or sulfatase in the presence or absence of the β-glucuronidase inhibitor d-saccharic,1-4 lactone. Thus, the aglycones released by β-glucuronidase, which could be inhibited by d-saccharic,1-4 lactone, were used to calculate the glucuronide concentration in urine and plasma. Similarly, the aglycones released after digestion with sulfatase and d-saccharic,1-4 lactone were used to calculate isoflavone sulfate concentrations. The results shown in Figure 6 were obtained with the use of these enzymes and the inhibitor, and they show that the profile of isoflavones in plasma differed.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>$t_{max}$ (h)</th>
<th>$C_{max}$ (nmol/L)</th>
<th>$t_{1/2z}$ (h)</th>
<th>AUC (nmol · h/L)</th>
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<tr>
<td>Daidzein</td>
<td>5.50 ± 0.49</td>
<td>298 ± 32</td>
<td>3.4 ± 0.3</td>
<td>2287 ± 252</td>
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<tr>
<td>Genistein</td>
<td>4.40 ± 0.43</td>
<td>654 ± 46</td>
<td>7.90 ± 0.72</td>
<td>8682 ± 942</td>
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$\bar{x} ±$ SEM, $t_{max}$, time to maximum plasma concentration; $C_{max}$, maximum plasma concentration achieved; $t_{1/2z}$, apparent half-life; AUC, area under the curve.
FIGURE 4. Mean (± SEM) plasma concentrations of genistein and daidzein in 12 individual subjects (● and ○, respectively) compared with those of plasma pooled from all 12 subjects (□ and △, respectively). Values represent determinations by liquid chromatography–mass spectrometry of aglycones generated after hydrolysis with β-glucuronidase.

FIGURE 5. Plasma aglycone concentrations of genistein (●) and daidzein (○) in pooled samples after enzymatic hydrolysis with β-glucuronidase (glucuronides) or sulfatase (sulfates). Values are the means of triplicate liquid chromatography–mass spectrometry determinations of aglycones generated after hydrolysis with β-glucuronidase or sulfatase.

substantially from that in urine. The following features were evident: 1) the percentages of genistein or daidzein excreted into urine as the sulfate conjugates were one-fifth to one-half less than those in plasma ($P < 0.05$), and 2) the percentages of genistein or daidzein excreted into urine as the glucuronide conjugates were 1.5–2.5 times those in plasma ($P < 0.05$).

On closer examination of these data, the sum of the percentages of aglycones released from sulfate conjugates and glucuronide conjugates (the cross-hatched bars labeled “S + G” in Figure 6) did not equal the total aglycone value (black bars labeled “T” in Figure 6) as determined with the sulfatase-β-glucuronidase preparation, indicating that conjugates other than those recognized by β-glucuronidase and sulfatase must be present in both the urine and the plasma. This was especially true for plasma, in which > 40% of the total isoflavone equivalents could

FIGURE 6. Mean (± SEM) percentages of total plasma and urine aglycones generated after hydrolysis with sulfatase (S), β-glucuronidase (G), or sulfatase and β-glucuronidase (T, for total aglycones); $n = 5$. S + G, the sum of the S and G bars; Seq, total aglycones generated after sequential hydrolysis with β-glucuronidase followed by sulfatase. *Significantly different from plasma, $P < 0.05$.

### Table 2

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<tr>
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<th>$t_{\text{max}}$</th>
<th>$C_{\text{max}}$</th>
<th>$t_{1/2}$</th>
<th>AUC</th>
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<tbody>
<tr>
<td></td>
<td>$h$</td>
<td>nmol/L</td>
<td>$h$</td>
<td>nmol·h/L</td>
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<tr>
<td>Glucuronides</td>
<td></td>
<td></td>
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<td></td>
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<td>Daidzein</td>
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<td>2587</td>
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<td>Genistein</td>
<td>6.0</td>
<td>499</td>
<td>8.4</td>
<td>7987</td>
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<td>Sulfates</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Daidzein</td>
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<td>3.1</td>
<td>1690</td>
</tr>
<tr>
<td>Genistein</td>
<td>4.5</td>
<td>141</td>
<td>5.7</td>
<td>1559</td>
</tr>
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$\text{AUC} = \text{area under the curve}$. $t_{\text{max}}$, time to maximum plasma concentration; $C_{\text{max}}$, maximum plasma concentration achieved; $t_{1/2}$, apparent half-life; $AUC$, area under the curve.
not be accounted for by summing the percentages of aglycones released after β-glucuronidase digestion and sulfatase digestion. In an effort to further study these conjugates, samples were digested sequentially with β-glucuronidase and sulfatase as described above. The results from this sequential digestion are indicated by the gray bars labeled “Seq” in Figure 6. As can be seen, these data more closely approximated the total concentrations of genistein and daidzein as determined by digestion with the sulfatase-glucuronidase preparation and presumably represent most of the conjugated forms of genistein and daidzein, including pure and mixed conjugates.

DISCUSSION
The health effects of soy and soy phytochemicals are currently being researched in several laboratories. There are many reports showing that soy isoflavones can alter cellular events important in either the promotion or prevention of certain diseases (13–16). Genistein and daidzein are the 2 most abundant isoflavones in soy and the most widely studied.

Studies in this area have focused either on purified genistein or daidzein or on soy products that contain the isoflavone glycosides genistin or daidzin. Because the aglycones have been shown to have effects in cell systems devoid of substantial conjugating enzymes, it has been widely assumed that the biologically active molecules are either the parent genistein or daidzein or their unconjugated metabolites. However, there are reports suggesting that the conjugates may either have biological activity themselves or serve as excellent sources of biologically active compounds at or within target cells. For example, daidzein-7,4’-di-O-sulfate competitively inhibits sterol sulfatase in hamster liver microsomes, whereas daidzein does not (17). Similarly, sulfate conjugates of endogenous steroids are thought to possess biological activity and to be an important source of free cellular steroids after sulfatase hydrolysis (18). It is possible, therefore, that sulfated isoflavones are active in vivo or are a primary source of free cellular aglycones after enzymatic hydrolysis in target tissues. Genistein glucuronides may also be active in vivo because they have been shown to have weak estrogenic activity and can activate human natural killer cells in vitro (19). It is also possible that the glucuronide conjugates of isoflavones are a source of free cellular aglycones, because deglucuronidation of estradiol- or estrone-3β-d-glucuronide takes place in kidney and liver lysosomes and microsomes in Syrian hamsters (20). Thus, the biological importance of the isoflavone conjugates may be multifaceted, ranging from the inactivation and excretion of dietary phytoestrogens to the regulation of specific biological processes either by direct action or by serving as an immediate source of aglycones within target tissues. Because the bioactivity of isoflavones is thought to be an integral component of soy’s health effects and because this bioactivity may be linked to the kinetics of the conjugates, we studied isoflavone pharmacokinetics in more detail.

Several pharmacokinetic studies of isoflavones in humans after soy consumption have been conducted (12, 21–29), but as far as we are aware, this is the first report to determine the plasma pharmacokinetics of genistein and daidzein sulfates in persons who consumed a soy meal. In addition, we characterized the conjugates of genistein and daidzein in both the plasma and urine. We found that the pharmacokinetics of genistein differ from those of daidzein. Kinetic analysis showed that glucuronide and sulfate conjugates of daidzein were cleared faster than were genistein conjugates and that the total concentration of circulating genistein (AUC) was ≈2 times that of daidzein. These results may have arisen because 1) the intake of genistein is 40% greater than that of daidzein, or 2) the peak excretion rate for daidzein is 2- to 3-fold that of genistein (10, 12). The shorter clearance time of the sulfate conjugates of genistein and daidzein, compared with that of genistein glucuronide, may be important if the sulfates are bioactive, because this would affect the duration of action. An understanding of the pharmacokinetics, combined with knowledge of the mechanisms of action and with dose-response data, could be used to determine the frequency of dietary soy-protein intake necessary to maintain a given biological effect.

Concentrations of daidzein sulfate in the plasma were 167% higher than those of genistein sulfate. The reasons for this are not clear, especially because the percentages of genistein and daidzein sulfates excreted in the urine were approximately the same (3–4%). This may reflect relative differences in urinary and biliary excretion of the isoflavones (3, 8). However, if sulfate conjugates play any role in the bioactivity of isoflavones, the higher circulating concentrations of daidzein sulfate may provide a larger pool for biological effects. Furthermore, one of the most important factors not yet determined, but currently being investigated with the use of animal models in our laboratory, is the tissue concentration of these isoflavone aglycones and conjugates.

Interpretation of the data from sequential digestion of the conjugates is only speculative. However, one possible explanation is that β-glucuronidase and sulfatase recognize mono- and diglucuronide conjugates and mono- and disulfate conjugates, respectively, and can cleave these bonds. This generates free aglycones that are detected by MS and used as the measure or indicator of the conjugate. However, hydrolysis of mixed conjugates by either enzyme alone will not generate aglycones, and thus these conjugates go undetected. Combining the β-glucuronidase and sulfatase generates aglycones from mixed conjugates and allows MS detection and quantitation of these conjugates. The data in Figure 6 suggest the presence of a significant concentration of mixed conjugates in plasma but not in urine. The role of mixed conjugates, especially in the bioactivity of isoflavones, is unknown. Further investigation is necessary to determine the mechanisms by which sequential digestion occurs, the structure of the mixed conjugates, and the biological role, if any, of mixed conjugates.

In summary, there are 2 primary findings in this report. First, we determined the pharmacokinetics of genistein and daidzein sulfates in men and women after consumption of a beverage containing SPI. The sulfate conjugates represented ≈10% and 25% of the total genistein and daidzein equivalents in plasma, respectively. There were substantial differences in the plasma dynamics of genistein and daidzein sulfates. The maximum concentration of daidzein sulfate was higher than that of genistein sulfate after the same time after consumption of the beverage, but the concentration of daidzein sulfate decreased faster than that of genistein sulfate. The significance of the plasma pharmacokinetics is unknown, but the sulfate conjugates are probably important in regulating the bioactivity of daidzein and genistein at the target tissue.

Second, we used a sequential enzyme digestion to further assess isoflavone conjugates. We speculate that there are 2 major types of genistein and daidzein conjugates in the plasma of persons who consume a soy meal: 1) those in which either or both conjugation sites are glucuronidated or sulfated (pure conjugates) and 2) mixed conjugates.
conjugates in which one site is glucuronidated and the other is sulfated. The profile of the daidzein and genistein conjugates in the 24-h urine sample differed substantially from that of plasma collected 4 h after consumption of a soy meal, suggesting that renal clearance may involve metabolic processing of isoflavone conjugates.

REFERENCES