Serum Levels and Metabolic Clearance of the Isoflavones Genistein and Daidzein in Hemodialysis Patients

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Abstract. Genistein and daidzein are biologically active isoflavones that are especially abundant in soybeans. After intestinal absorption, circulating genistein and daidzein are eliminated primarily by the kidneys. This study was undertaken to assess the metabolism of genistein and daidzein in patients with end-stage renal disease (ESRD) on hemodialysis therapy, and to test whether this treatment modality can replace the lack of kidney function, with respect to the elimination of the isoflavones. Twenty-three hemodialysis patients and 10 healthy subjects were studied. While consuming a self-selected low isoflavone diet, baseline blood levels were undetectable in eight of 10 healthy subjects and in 14 of 23 dialysis patients. The remaining participants had detectable levels, with the nine dialysis patients displaying much higher blood concentrations than the two healthy control subjects. After the evening intake of one dose of an isoflavone-rich soy protein isolate drink, the early morning blood levels of genistein and daidzein were higher in seven dialysis patients than in eight healthy subjects (genistein 1271 ± 321 versus 425 ± 104, \( P < 0.05 \); daidzein 1304 ± 352 versus 292 ± 78, \( P < 0.05 \)). The blood clearance of the isoflavones was studied in two healthy subjects and in three dialysis patients. Genistein and daidzein were eliminated within 2 d in the healthy subjects, but had not returned to baseline in two of three ESRD patients, 7 d after intake. The half-life of both compounds was estimated to be 10-fold longer in the ESRD patients than in the healthy subjects. Finally, genistein and daidzein levels were measured before and after dialysis in five patients, both while on their regular diet and after one dose of a soy protein isolate drink. In both instances, the dialysis treatment did not affect the blood isoflavone levels. In conclusion, approximately one-third of hemodialysis patients eating the standard American renal diet experience high blood levels of the isoflavones genistein and daidzein, while the remaining two-thirds have undetectable levels. After ingestion of isoflavone-rich food such as soy products, all patients have detectable levels that remain very high for several days due to lack of renal excretion.

The isoflavones are a large group of heterocyclic phenols with chemical structure similar to estradiol. They are variably abundant in edible plants predominantly in legumes and have received considerable attention lately, both from the general population and the scientific community, due to a number of documented and postulated biologic effects in humans, including modulation of the reproductive function and prevention of cardiovascular disease, osteoporosis, and cancer (reviewed in references (1 and 2)). Genistein and daidzein are the most abundant and the best-characterized isoflavones, and are particularly abundant in soy including its food products (3,4).

Once absorbed, a small fraction of the isoflavones circulates systemically in their unconjugated form. However, the largest fraction undergoes glucuronidation and sulfatation by the gut wall and the liver (5), followed by enterohepatic circulation. Both conjugated and unconjugated isoflavones are eliminated by kidneys and liver, primarily as glucuronides and sulfates (6,7). The urinary excretion of the isoflavones correlates with certain diets (8), and a strong correlation has been observed between soy consumption and urinary isoflavonoid levels in multiethnic populations (9).

In renal failure, the ability of the kidneys to excrete the major isoflavone fraction is reduced. It is not known whether in renal failure other organs and tissue can compensate for the reduced urinary excretion of the isoflavones by decreasing their absorption or increasing their extrarenal elimination. Furthermore, it is not known whether dialysis therapy is capable of replacing kidney function, with respect to isoflavone clearance. If, as postulated by us, the isoflavones accumulate in uremia, they may cause a variety of biologic effects due to their ability to affect gonadotropin secretion (10,11), lipid metabolism (12), oxidative stress (13), bone metabolism (14), and carcinogenesis (15,16).

This study examined the circulating levels and the metabolic clearance of genistein and daidzein in end-stage renal disease (ESRD) patients and in healthy subjects. We also examined the effect of hemodialysis therapy on the circulating levels of these isoflavones.

Materials and Methods

Experimental Subjects

Adult patients with ESRD undergoing chronic maintenance hemodialysis and healthy subjects were studied. Twenty-three clinically stable patients were recruited from the 124 individuals enrolled in the
chronic hemodialysis program of the University of Kentucky. The patients were dialyzed for 4 h three times weekly using cuprophane (Gambro, Lund, Sweden), cellulose acetate (Althien, Miami Lake, FL), or polysulfone membrane dialyzers (Fresenius, San Francisco, CA). The dialysis surface area ranged from 0.9 to 1.8 m², and the blood flow was 300 ml/min with dialysate flow of 600 ml/min. Table 1 reports the mean value of important clinical variables in the whole population, and Table 2 gives individual values in 11 of these patients who were randomly selected for further investigation involving intake of standardized amounts of isoflavone-rich soy protein isolate (SPI). Additionally, 10 healthy subjects (five men and five women, mean age 38, body mass index 24.1 ± 1.9) volunteered for the study. None of the participants suffered from gastrointestinal problems that could interfere with isoflavone absorption, including acute gastrointestinal illness, the use of antibiotics within the past 3 mo, chronic malabsorption syndrome, chronic hepatitis, and liver cirrhosis. Furthermore, none of the participants suffered from uncompensated heart failure, unstable coronary artery disease, respiratory illness, severe hyperparathyroidism, aluminum toxicity, cancer, drug addiction, ethanol abuse, or smoking of more than 10 cigarettes per day. All candidates were interviewed by a licensed dietitian. Participants were instructed to follow their routine diet with the exception of carefully avoiding isoflavone-rich food for 1 wk (healthy subjects) and 2 wk (patients) before their participation in the study. Informed consent approved by the University of Kentucky Institutional Review Board was read and signed by all subjects.

**Study Protocols**

Baseline blood levels of the isoflavones were measured in all participants, while on the self-selected isoflavone-poor diet. A single baseline blood sample was collected from the 10 healthy subjects. Conversely, the 23 dialysis patients had baseline samples collected weekly for 4 consecutive weeks, due to the suspicion that these patients may experience day-to-day fluctuations of the circulating level of the isoflavones (17). The healthy subjects and the 14 dialysis patients who were routinely dialyzed in the morning were phlebotomized between 7 a.m. and 8 a.m. while fasting. The remaining nine dialysis patients who were dialyzed in the afternoon, were phlebotomized between 11 a.m. and 12 p.m., without restrictions of the eating schedule. The four baseline samples from the dialysis patients were analyzed individually in eight randomly selected patients, while they were pooled into one specimen per patient for single baseline determinations in the remaining 15 subjects.

To investigate the effect of dietary intake of the isoflavones on their blood levels, these phytochemicals were measured in the eight healthy subjects and three patients after ingestion of one dose of SPI as above. In these patients, blood samples were obtained in the morning before ingestion of the SPI (baseline), then 8 h, and 2, 5, and 7 d after the SPI load. The healthy subjects were sampled before and 4, 6, 8, 16, 24, and 48 h after the SPI load. A urine sample was obtained from the latter subjects at the time of each blood collection.

To test the effect of dialysis treatment on the blood levels of the isoflavones, blood samples were collected from five dialysis patients immediately before and at the end of a routine dialysis session. The blood sampling was repeated twice in each patient, i.e., while on the isoflavone-poor routine diet and after an evening SPI load, as described above.

Blood was drawn into Vacutainers (Becton Dickinson, Franklin, NJ), allowed to clot at 21°C for 30 min, and centrifuged for 15 min at 10°C and 1000 × g. The supernatant serum was stored at −70°C until analyzed. The urine samples were collected in plain disposable containers and were kept at −70°C until analyzed.

**Analysis of Isoflavones from Serum and Urine**

Extraction and enzymatic hydrolysis of isoflavones from the urine was carried out with minor modifications from our previously described method (17). In brief, frozen urine was equilibrated at room temperature, vortex-mixed, and centrifuged at 850 × g for 20 min. Two milliliters of clear urine was mixed with 0.5 ml of 0.5 M triethylamine acetate buffer, pH 7.0, and 20 μl of flavone (120 ppm in EtOH 96%; Aldrich, Milwaukee, WI) as internal standard. The samples were incubated for 1 h at 37°C with 10 μl of β-glucuronidase (200 U/ml at 25°C; Boehringer Mannheim, Indianapolis, IN) and 10 μl of arylsulfatase (5 U/ml at 25°C; Boehringer Mannheim). Subsequently, the isoflavones were extracted three times into 2 ml of diethyl ether (ACS-certified). The combined ether fractions were evaporated to dryness under a stream of nitrogen and were redissolved in 140 μl of methanol and 60 μl of 0.2 M sodium acetate buffer, pH 4.0. Samples were analyzed immediately by injecting 20 μl into the HPLC system or stored at −20°C until analyzed.

Extraction and enzymatic hydrolysis of isoflavones from human serum was performed as described previously (18). In brief, 1.0 ml of human plasma equilibrated to room temperature was mixed with 0.25 ml of 0.5 M triethylamine acetate, pH 7.0, 80 μl of β-glucuronidase (200 U/ml), 80 μl of arylsulfatase (5 U/ml), and 20 μl of flavone (120 ppm in EtOH 96%) followed by stirring for 17 h at 37°C in a sealed container. The samples were mixed with 0.25 ml of 10% aqueous TCA and extracted three times with 2 ml of ethyl acetate (ACS-certified). After centrifugation, the organic phases were combined, dried under nitrogen, redissolved in 100 μl of methanol by vortexing, spiked with 100 μl of 0.2 M acetic acid buffer, pH 4, and sonicated for 30 s. The samples were analyzed immediately by injecting 100 μl into the HPLC system or stored at −20°C.

**Table 1. Clinical data and serum biochemical results in chronically dialyzed patients**

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>HD (mo)</th>
<th>BMI (kg/m²)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>CO₂ (mM)</th>
<th>Cholesterol (mg/dl)</th>
<th>Kt/V</th>
<th>nPCR (g/kg per d)</th>
<th>RRF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 ± 3</td>
<td>14 M 9 F</td>
<td>24 ± 4</td>
<td>23.6 ± 0.8</td>
<td>62 ± 3</td>
<td>10.0 ± 0.6</td>
<td>3.7 ± 0.1</td>
<td>21.3 ± 0.7</td>
<td>163 ± 10</td>
<td>1.50 ± 0.05</td>
<td>0.99 ± 0.05</td>
<td>0.21 ± 0.12</td>
</tr>
</tbody>
</table>

* Mean ± SEM values for the whole patient population (n = 23). HD, hemodialysis; BMI, body mass index; BUN, blood urea nitrogen; nPCR, normalized protein catabolic rate; RRF, residual renal function.
Table 2. Selected patients challenged with oral isoflavones

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Renal Dz</th>
<th>HD (mo)</th>
<th>MD/SA (m²)</th>
<th>BMI</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>CO₂ (mM)</th>
<th>Cholesterol (mg/dl)</th>
<th>Kt/V</th>
<th>nPCR (g/kg per d)</th>
<th>RRF (ml/min)</th>
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<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>LN</td>
<td>70</td>
<td>PS/1.2</td>
<td>17.3</td>
<td>49</td>
<td>13.6</td>
<td>3.6</td>
<td>18</td>
<td>187</td>
<td>1.46</td>
<td>0.75</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>F</td>
<td>DM</td>
<td>30</td>
<td>PS/0.9</td>
<td>23.6</td>
<td>77</td>
<td>7.9</td>
<td>3.7</td>
<td>19</td>
<td>172</td>
<td>1.57</td>
<td>0.89</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>M</td>
<td>DM</td>
<td>48</td>
<td>PS/1.2</td>
<td>34.8</td>
<td>49</td>
<td>6.8</td>
<td>3.5</td>
<td>27</td>
<td>164</td>
<td>1.21</td>
<td>1.07</td>
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</tr>
<tr>
<td>4</td>
<td>70</td>
<td>M</td>
<td>HTN</td>
<td>29</td>
<td>CA/1.8</td>
<td>25.6</td>
<td>74</td>
<td>14.8</td>
<td>4.0</td>
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<td>98</td>
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<tr>
<td>5</td>
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<td>M</td>
<td>DM</td>
<td>14</td>
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<td>9.4</td>
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<td>25</td>
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<td>10.6</td>
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<td>0.79</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>M</td>
<td>HTN</td>
<td>27</td>
<td>C/1.3</td>
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<td>8.7</td>
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<td>0</td>
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<td>M</td>
<td>DM</td>
<td>30</td>
<td>CA/1.8</td>
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<td>40</td>
<td>12.1</td>
<td>3.6</td>
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<tr>
<td>9</td>
<td>71</td>
<td>M</td>
<td>DM</td>
<td>38</td>
<td>CA/1.8</td>
<td>28.3</td>
<td>85</td>
<td>12.3</td>
<td>4.1</td>
<td>21</td>
<td>156</td>
<td>1.44</td>
<td>1.13</td>
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<td>67</td>
<td>M</td>
<td>DM</td>
<td>3</td>
<td>PS/1.2</td>
<td>28.6</td>
<td>63</td>
<td>11.3</td>
<td>3.8</td>
<td>22</td>
<td>222</td>
<td>1.74</td>
<td>0.79</td>
<td>2.43</td>
</tr>
</tbody>
</table>

* These 10 patients are from the population of 23 described in Table 1. Dz, disease; MD, type of membrane dialyzer; SA, surface area; LN, lupus nephritis; PS, polysulfone; DM, diabetes mellitus; HTN, hypertension; CA, cellulose acetate; C, cuprophane.

Chromatographic Conditions

HPLC analysis was performed using a system GOLD instrument #338 (Beckman, Fullerton, CA) and a NovaPak C18 (150 × 3.9 mm inner diameter; 4 μm) reversed-phase column (Waters, Milford, MA) coupled to an Adsorbosphere C18 (10 × 4.6 mm inner diameter; 5 μm) direct-connect guard column (Alltech, Deerfield, IL). Elution was performed at a flow rate of 0.8 ml/min with the following linear gradient: A = acetic acid/water (10:90; vol/vol), B = methanol/acetonitrile/dichloromethane (10:5:1; vol/vol/vol); B in A (vol/vol): 5% for 5 min, from 5 to 45% in 20 min, from 45 to 70% in 6 min, and from 70 to 5% in 3 min with equilibration for 15 min before subsequent injection. Analytes were monitored by photo-diode array detection at 260 and 280 nm simultaneously during the entire HPLC run. Peaks were scanned between 190 and 400 nm for identification purposes and quantified using peak areas after calibration with authentic standards and adjustment for internal standard recovery. The detection limit for identification of the genistein and daidzein peaks by ultraviolet scans was 35.8 and 62.8 nM, respectively, based on a signal-to-noise ratio of 5. Final isoflavone excretion in urine was expressed in nanomoles per mg creatinine (nmol/mg). Urine creatinine concentrations were determined by a test kit based on the Jaffe reaction at 260 and 280 nm simultaneously during the entire HPLC run. Peaks were scanned between 190 and 400 nm for identification purposes and quantified using peak areas after calibration with authentic standards and adjustment for internal standard recovery. The detection limit for identification of the genistein and daidzein peaks by ultraviolet scans was 35.8 and 62.8 nM, respectively, based on a signal-to-noise ratio of 5. Final isoflavone excretion in urine was expressed in nanomoles per mg creatinine (nmol/mg). Urine creatinine concentrations were determined by a test kit based on the Jaffe reaction (Sigma catalog no. 555, St. Louis, MO). The genistein and daidzein half-lives were determined for the individual experimental subjects by plotting the difference in the log (concentration of the isoflavones – the baseline concentration) versus time. On the basis of first-order disappearance kinetics, the half-lives were estimated from the terminal linear part of the log concentration-time profile (19).

Other Biochemical Measurements

Serum albumin, total protein, total cholesterol, alanine aminotransferase, total bilirubin, carbon dioxide, blood urea nitrogen, and creatinine were measured using a Hitachi 747 Automated Chemistry Analyzer (Boehringer Mannheim).

Statistical Analyses

All results are given as the mean ± SEM. Differences were calculated using the two-tailed t test for paired and independent samples, and with one-way and two-way ANOVA. The proportions of detectable measurements in the two groups were compared using the two-tailed Fisher exact test for 2 × 2 contingency tables. All computations were performed with the SPSS software package (SPSS, Inc., Chicago, IL), using an IBM-clone PC.

Results

Eight randomly selected patients had each of the four baseline samples tested separately. As shown in Figure 1, serum genistein and daidzein were undetectable in three patients, but were detectable and varied substantially from week to week in the other five patients. Figure 2 shows baseline concentrations of genistein and daidzein in each participant. In the healthy subjects, the determinations were done in single specimens and in the dialysis patients they were done in pooled sera from four weekly baseline blood collections. The isoflavone blood levels displayed a mixed distribution in the 23 hemodialysis patients, with 55 to 65% of the patients having undetectable levels and 1/4 to 1/3 showing levels above 200 nM. The frequency of undetectable baseline levels was not different between the hemodialysis patients and the healthy subjects. Between-groups statistical comparison of the measurable levels was not done due to the very small number of such values in the healthy control subjects. Additionally, Figure 2 shows that the time of blood collection and the intake of isoflavone-poor food did not appear to affect the baseline isoflavone levels in the dialysis patients, since genistein and daidzein levels were comparably distributed in fasting early morning blood samples (Figure 2, filled circles) and midday nonfasting samples (Figure 2, filled triangles). The daidzein metabolites equol and O-desmethylangolensin and the minor soy isoflavone glycitein were undetectable in the above samples.

Eight to twelve hours after consumption of one dose of the SPI drink, blood levels of genistein and daidzein had increased markedly in both hemodialysis patients (genistein 1271 ± 321 nM and daidzein 1304 ± 352 nM) and healthy subjects (genistein 424 ± 104 nM and daidzein 292 ± 78) (Figure 3). In both populations, the post-load levels were significantly higher than at baseline (P < 0.001). Additionally, the post-load blood levels and the difference between the post-load and
baseline levels were significantly higher in the hemodialysis patients than in the healthy subjects (P < 0.05) (Figure 3). Metabolic clearance levels of genistein and daidzein in healthy individuals are illustrated in Figure 4. Genistein and daidzein were detectable 4 h after consumption of one dose of SPI, reached peak concentrations after 6 to 8 h after intake, and had returned to undetectable levels within 2 d after the oral load. Urinary excretion of genistein and daidzein followed a similar pattern (Figure 4). In the hemodialysis patients (Figure 5), the highest levels of genistein and daidzein were also detected 8 h after the oral load. However, in these patients the disappearance rate of the isoflavones was extremely slow, and 7 d after the oral load genistein and daidzein were eliminated completely in one patient, by 96 and 89%, respectively, in the second patient, and just by 67 and 36% in the third. The half-lives of genistein and daidzein were calculated in the two healthy subjects and three patients, based on the above blood concentration profiles. In the two healthy subjects, the half-lives were 3.5 and 6 h for genistein and 3.5 and 3.5 h for daidzein. Conversely, in the renal failure patients, the half-life of genistein was 39, 43, and 53 h, and that of daidzein 30, 44, and 99 h.

To test whether standard dialysis treatment affects the blood levels of genistein and daidzein, blood samples were obtained immediately before and after hemodialysis treatment in five dialysis patients. In each patient, the test was performed while on routine diet and, again, during a dialysis treatment that was preceded 12 to 16 h by intake of one serving of SPI. The circulating levels of genistein and daidzein were not affected by dialysis treatment, regardless of whether the patients had been on their routine diet (genistein: 60 ± 49 nM versus 91 ± 61 nM; daidzein 170 ± 126 nM versus 0.160 ± 114 nM) or whether they had received the oral dose of SPI before the dialytic treatment (genistein: 714 ± 186 nM versus 656 ± 223 nM; daidzein: 961 ± 477 nM versus 1036 ± 391 nM).

Discussion
The present study is the first to report the blood levels of the isoflavones genistein and daidzein in patients with ESRD eating the standard American diet, the metabolic fate of these phytochemicals following intake of a single serving of an isoflavone-rich soy product, and their lack of clearance with conventional hemodialytic therapy.

Figure 1. Serum levels of genistein and daidzein measured weekly in eight hemodialysis patients who were on self-selected standard American renal diets. The numbers next to the symbols identify each case with one of the patients of Table 2.

Figure 2. Individual baseline serum levels of genistein and daidzein in 10 healthy subjects and 23 hemodialysis patients. The determinations in the healthy subjects were performed on single specimens, whereas in the hemodialysis patients they were done on pooled sera from four weekly blood collectors. ●, dialysis patients bled between 7 and 8 a.m., while fasting; ▲, dialysis patients bled between 11 and 12 a.m. without restrictions of the meal schedule. The healthy subjects (○) were bled between 7 and 8 a.m., while fasting.
The baseline blood levels of genistein and daidzein were statistically not different between dialysis patients and healthy subjects who consumed the standard isoflavone-poor American diet. Indeed, the majority of the participants in the two populations had undetectable baseline levels of genistein and daidzein. The presence of measurable levels in a fraction of the healthy control subjects and of the ESRD patients could be due to unintentional dietary intake of soy. In fact, North American manufacturers of processed food add variable amounts of soy to their products, so that consumers can be exposed unknowingly to the isoflavones. Additionally, the mixed distribution of the baseline levels may be due to between-subject differences in metabolic handling of the isoflavones. In fact, it was shown that healthy subjects exposed to the same isoflavone dose have variable blood levels and urinary recovery of these phytochemicals (17,20,21). Differences in dietary habits, the gut flora, intestinal transit time, and redox level are believed to be responsible for this variability among healthy subjects(22), and they are likely to play a similar role in patients with ESRD. Furthermore, ESRD patients are often exposed to antibiotics that can alter the intestinal flora, thus changing the intestinal handling of the isoflavones (23). Another possibility is that the rate of degradation of the isoflavones in the target tissue varies among subjects. The latter mechanism is probably irrelevant.
for the isoflavone levels of healthy subjects who can eliminate the phytochemicals efficiently via the kidneys, but it may affect substantially the levels of patients who tend to accumulate the isoflavones because of lack of renal function.

The time of the blood collections and the ingestion of food on collection day did not seem to affect the baseline concentrations in the ESRD population, since the results were comparable in patients studied in the early morning while fasting or around noon time after intake of food. This suggests that as long as the diet is isoflavone-free or isoflavone-poor, baseline measurements in the ESRD patients can be obtained at the time of dialysis treatment, regardless of food intake.

The five subjects that had detectable levels in four separately tested baseline samples showed considerable variation of the isoflavone concentration from week to week. This is consistent with observations in healthy subjects(17), and it suggests that measurement of genistein and daidzein in single random samples in ESRD patients may not reflect with sufficient accuracy the prevailing isoflavone levels. Pooling of sample from at least three weekly drawings may be a reasonable compromise between the cost of the test and the need for representative values for a given patient. Additionally, it is noted that the fluctuations over time of the baseline concentrations of genistein and daidzein are not always consensual. A possible explanation of this finding is that the isoflavones have distinct degradation pathways that depend, to a good extent, on the intestinal flora (21). Thus, variations of the intestinal flora over time may favor the degradation of one phytochemical over the other, in turn affecting their blood levels.

Statistical comparison limited to the measurable baseline levels of genistein and daidzein was not possible due to the small number of such values in the control group. However, inspection of the available data (Figure 2) shows a clear tendency for the ESRD patients to have higher genistein and daidzein levels (60 and 1600 nM) than the healthy control subjects (36 and 78 nM). Additionally, challenge with one dose of isoflavone-rich SPI resulted in post-load isoflavone levels that were significantly higher in the ESRD patients than in the healthy control subjects. The tendency for both baseline and post-load levels to be higher in the ESRD patients can be attributed to lack of renal clearance in this population. This possibility is corroborated by comparison of the rates of elimination of the isoflavones in two healthy subjects and three ESRD patients, following challenge with a single dose of SPI. Intake of SPI by the healthy control subjects resulted in peak blood levels of the isoflavones in the hundred nanomolar range, and in clearance of these chemicals from the circulation within 48 h, similar to previously reported studies (17,24). Peak urinary concentrations in these two subjects were achieved after 6 and 8 h, suggesting that rapid renal excretion of the isoflavones during the early post-load period affects negatively the peak blood levels of healthy individuals. Among the ESRD patients, the intestinal absorption of the isoflavones appeared normal since peak levels were achieved 4 to 8 h after oral challenge with SPI, as in the healthy subjects. However, the clearance of the isoflavones was very slow in these patients, with half-lives that were 10-fold higher than in the healthy subjects. The mechanism(s) maintaining the residual low level of clearance of the isoflavones in ESRD are not known. Genistein undergoes enterohepatic recirculation (5), and it is likely that small amounts of the chemicals are lost in the feces during each enteric pass (25). Alternatively, genistein and daidzein may be metabolized by the peripheral tissues, in a manner similar to the chemically related synthetic compound ipriflavone (26). In addition, it is possible that ESRD patients have sufficient residual kidney function to maintain low rates of renal excretion and/or degradation of the isoflavones.

The wide variability of the peak levels in both healthy

Figure 5. Metabolic clearance of genistein and daidzein in three hemodialysis patients (solid lines) and two healthy subjects (dotted lines). Levels of the isoflavones were measured in the serum at baseline and 8, 24, 48, 72, 96, 120, and 168 h after ingestion of one dose of soy protein drink. The gray boxes indicate performance of hemodialysis. The numbers next to the symbols identify each case with one of the patients of Table 2.
subjects and ESRD patients is in good agreement with reports by other investigators (25). As in the case of the baseline isoflavone levels, this variability may be attributed to individual dietary habits, intestinal flora, intestinal motility, and redox activity. Additionally, in our study the SPI dose was not adjusted for body mass.

The failure of standard hemodialysis therapy to remove the isoflavones is not surprising, since approximately 90% of the isoflavones circulate as glucuronic and sulfuric acid conjugates (27) with molecular weights in excess of 330 daltons. Thus, based on the molecular size, the conjugated isoflavones are at the low end of the poorly dialyizable “middle molecule pool” (molecular weight 300 to 12,000 daltons; reference 28). Furthermore, approximately 50% of unconjugated genistein and 80% unconjugated daidzein circulate bound to albumin in healthy subjects (29), which makes them fully unavailable for dialysis.

The clinical significance of accumulation of the isoflavones in the presence of renal failure is unknown. This uncertainty is due primarily to the only very recent development of accurate and relatively rapid methods for the measurement of isoflavones in blood and to the scant information and much controversy existing about the biologic activity of conjugated isoflavones. Two studies have measured the free and conjugated isoflavones in human blood and have reported that the cumulative free and sulfated fractions in the blood of healthy subjects are 3 to 9% for genistein and 10 to 15% for daidzein (30,31). Conversely, the relative amounts of glucuronated, sulfated, and free isoflavones in the blood of ESRD patients are not known. There is good evidence that many tissues contain sulfatases capable of converting isoflavone-sulfates into biologically active free isoflavones (32). With respect to glucuronidation, in most cases this is associated with abolition or significant reduction in the biologic activity of the aglycone and it prompts the renal elimination of the biotransformed chemical. However, digoxin and morphine were shown to be effect is associated with stimulation of osteoblastic activity and that their accumulation in renal failure may favor without concomitant effects on osteoclastic bone resorption (14). This leads to the question of whether accumulation of isoflavones in renal failure affects the clinical presentation of renal osteodystrophy. Additionally, the production of tumor necrosis factor-α by monocyctic cells was suppressed in the same genistein-treated rats (14). Since the proinflammatory cytokines seem to participate to the pathogenesis of renal osteodystrophy (45,46), the question arises whether cytokine modulation by genistean affects uremic bone.

Additional research is needed to demonstrate whether an association exists between accumulation of isoflavones and the above-cited as well as other manifestations of ESRD. This, in turn, may lead to the formulation of new practices aimed at monitoring the dietary intake and the circulating levels of the isoflavones in renal failure.

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References

11. Goodwin NJ, Valenti C, Hall JE, Friedman EA: Effects of uremia...


22. Setchell KDR, Adlercreutz H: Mammalian lignans and phyto-


27. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentra-


30. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentra-


33. Osborne RJ, Joel SP, Slevin ML: Analgesic activity of morphine-


35. Faed EM: Decreased clearance of diflunisal in renal insuffi-

macol* 36: 165–174, 1989

37. Meffin PJ, Zilm DM, Veenaedal JR: Reduced clofibrate acid clearance in renal dysfunction is due to a futile cycle. *J Phar-
macol Exp Ther* 227: 732–738, 1983


42. Nestel PJ, Yamashita T, Sashara T, Ponery S, Dart A, Kome-

43. Knight DC, Eden JA: A review of the clinical effects of phy-

