

## Regulation of Phase II Enzymes by Genistein and Daidzein in Male and Female Swiss Webster Mice

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**ABSTRACT** The consumption of soy and soy isoflavones has been associated with a decreased risk of certain cancers. A factor contributing to this dietary chemoprevention is the activity of phase I and II biotransformation enzymes. This study evaluated the hypothesis that dietary soy isoflavones will increase hepatic and extrahepatic quinone reductase (QR), UDP-glucuronosyltransferase (UGT), and glutathione *S*-transferase (GST) phase II enzyme activities, under short-term feeding and basal (non-pharmacologic-induced) conditions. Male and female Swiss Webster mice were fed for 1, 3, 5, or 7 days of one of four treatments: control (casein AIN-93G) or control supplemented with flavone (positive control), genistein, or daidzein aglycones at 1,500 mg/kg of diet. QR activity was increased by daidzein in the liver, by both isoflavones in the kidney and small intestine, and by genistein in the heart. Genistein and daidzein slightly decreased UGT activities in some tissues. Liver GST activity was decreased by genistein in females. In contrast, genistein and daidzein increased kidney GST activity. In general, the greatest effects of isoflavones on phase II enzymes were observed in liver and kidney tissues, occurring at day 3, and peaking at day 5. Sex effects in the liver and kidney included females exhibiting higher QR activities and males exhibiting higher UGT and GST activities. In conclusion, individual soy isoflavones modulate phase II enzymes in mice under short-term feeding and basal conditions. This study provides insights into the actions of isolated isoflavones in mice.

**KEY WORDS:** • daidzein • genistein • phase 2 enzymes • soy

### INTRODUCTION

SOY ISOFLAVONES ARE MEMBERS of a large group of flavonoids that can occur in relatively high concentrations in certain fruits, vegetables, and grains.<sup>1</sup> In Japan and China, the consumption of soy protein is approximately 8–12 g/day, providing isoflavones (glycosides and aglycones) at 15–50 mg/day, which yields serum genistein concentrations of approximately 0.5–5  $\mu$ M from one soy-rich meal.<sup>1–5</sup> Soy protein and isoflavone consumption among adults of non-Asian descent in Western countries is typically  $\leq$  1–2 g/day and 1–3 mg/day, respectively.<sup>2</sup>

Epidemiologic studies have associated the consumption of soy-rich diets in Asian countries with a lower incidence of cardiovascular disease<sup>6</sup> and certain cancers.<sup>7</sup> Whole soy foods contain a number of bioactive components; the greatest scientific interest has been focused on the isoflavones genistein and daidzein because of their numerous biologic effects. Anticancer properties of isoflavones have been suggested to include mechanisms such as the inhibition of tyrosine kinases,<sup>8</sup> topoisomerase II,<sup>9</sup> angiogenesis,<sup>10</sup> 5 $\alpha$ -reductase,<sup>11</sup> growth factor-induced *c-fos* expression,<sup>12</sup> and cell growth.<sup>7</sup> Antioxidant activity,<sup>13</sup> promotion of apoptosis,<sup>14</sup> estrogen

receptor (ER) binding,<sup>15</sup> and modulation of steroid metabolism<sup>16</sup> are also thought to play roles in the health-promoting properties of soy.

Dietary chemoprevention by soy via modulation of the xenobiotic metabolizing system (phase I and II [PII] biotransformation enzymes) is another biological characteristic that merits further investigation. The phase I and PII biotransformation enzymes play central roles in the metabolism of endogenous compounds, xenobiotics, environmental pollutants, drugs, and food components. In general, a protective profile is thought to be represented by a decrease in a bioactivation of procarcinogens via phase I metabolism (cytochrome P450 enzymes) and/or an increase in PII metabolism to promote a faster detoxification and clearance of potentially carcinogenic intermediates.<sup>17</sup> The hydroxylated carcinogenic intermediates are detoxified by PII enzymes, such as quinone reductase (QR), glutathione *S*-transferase (GST), and UDP-glucuronosyltransferase (UGT), and are subsequently excreted from the body.

Several studies using cell<sup>18–20</sup> and animal<sup>21–23</sup> models indicate that isoflavones and soy proteins induce PII biotransformation enzymes. Thus, dietary components represent a mechanism to influence bioactivation and metabolism of both endogenous and exogenous compounds to promote health and reduce cancer risk. However, it is not known with certainty whether genistein and daidzein or other bioactive factors in soy are the components that modulate various PII enzymes in an animal model, the extent to which they

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respond under basal conditions (*i.e.*, not following pharmacologic or carcinogen-mediated induction), and the time course of response with short-term (<20 days) dietary exposure. Therefore, the objective of this study was to test the hypothesis that dietary genistein and daidzein increase the PII enzymes in various tissues of Swiss Webster mice under short-term feeding and basal conditions and to determine if there are isoflavone-specific, time-, and sex-related responses associated with the modulation of these metabolizing enzymes.

## MATERIALS AND METHODS

### *Chemicals and reagents*

Genistein and daidzein (aglycones) were purchased from Indofine Chemical Co., Inc. (Hillsborough, NJ, USA). Flavone (2-phenyl-4*H*-1-benzopyran-4-one), the backbone of the flavone class of flavonoids, was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were purchased from Sigma-Aldrich and Fisher Scientific International, Inc. (Hampton, NH, USA).

### *Animals*

Male and female Swiss Webster mice purchased from Charles River Laboratories, Inc. (Wilmington, MA, USA) were 8–10 weeks old, weighed 18–30 g, and were housed as sex-matched pairs in wire-bottom cages. The room temperature was maintained at 25°C with a 12-hour on and 12-hour off light cycle, and they were allowed free access to food and water. The animal study protocol was approved by the University of California Davis Animal Use and Care Committee, and the Association for Assessment and Accreditation of Laboratory Animal Care guidelines were followed.

### *Diets*

The control (casein AIN-93G) diet was purchased from Dyets, Inc. (Bethlehem, PA, USA). Treatment diets included flavone, genistein, or daidzein (all aglycones) at 1,500 mg/kg of diet. Flavone, a synthetic compound, was used as a positive control for the induction of the phase II enzymes.<sup>24</sup> Mice were fed the control (casein AIN-93G) diet for 1 week prior to feeding trials. The mice ( $n = 5$  per group) were randomly assigned to one of the four treatment groups: control (casein AIN-93G) or control supplemented with flavone, genistein, or daidzein at 1,500 mg/kg of diet and fed for up to 1 week. The mice were asphyxiated with CO<sub>2</sub> on day 1, 3, 5, or 7 after consumption of their diet.

### *Tissue preparation and collection*

Livers were perfused with phosphate-buffered saline (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, and 8.0 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4) and excised. The small intestine was excised and flushed with phosphate-buffered saline, and the enterocyte cells were removed by scraping with a microscope slide. The kidney, colon, heart, prostate, and stomach were removed and flushed with phosphate-buffered saline. All of the tissues were frozen in liquid nitrogen and stored at

–80°C. Microsomal and cytosolic fractions were obtained using standard differential centrifugation.<sup>25</sup> In summary, the tissues were homogenized in 4 mL of buffer (50 mM Tris-HCl, 0.154 M KCl, and 1 mM EDTA, pH 7.4)/g of tissue with a motor-driven pestle. The tissue homogenates were centrifuged at 10,000 *g* for 20 minutes at 4°C. The supernatants were centrifuged at 100,000 *g* for 60 minutes at 4°C. The cytosolic supernatants were dispensed into microcentrifuge tubes, frozen in liquid nitrogen, and stored at –80°C until further analysis. The microsomal pellets were removed and homogenized by hand with a pestle in 0.25 mL of buffer (0.1 M sodium phosphate, 1 mM EDTA, and 20% [vol/vol] glycerol, pH 7.4)/g of tissue. The homogenates were also dispensed into microcentrifuge tubes, frozen in liquid nitrogen, and stored at –80°C until further analysis.

### *Enzyme activity assays*

Cytosolic QR assays (activity given in nmol/minute/mg of cytosolic protein), to measure QR 1 activity, were analyzed in 96-well plates by following the conversion of glucose 6-phosphate + NADP to 6-phosphogluconate + NADPH by glucose 6-phosphate dehydrogenase, followed by NADPH + menadione to menadiol + NADP by QR 1 and the linked reduction of thiazolyl blue tetrazolium bromide spectrophotometrically at 600 nm for 10 minutes.<sup>26</sup> Cytosolic GST assays (activity given in  $\mu$ mol/minute/mg of cytosolic protein), to measure  $\alpha$ ,  $\mu$ , and  $\pi$  GST activities, were analyzed in cuvettes by following the conversion of the substrates glutathione and 1-chloro-2,4-dinitrobenzene to the glutathione-conjugated product spectrophotometrically at 340 nm every 30 seconds for 5 minutes.<sup>27</sup> Microsomal UGT assays (activity given in nmol/minute/mg of microsomal protein), using the substrate 3-methyl-2-nitrophenol, measured the conversion of UDP-glucuronic acid to *R-O*-glucuronide by phenolic-UGT. These assays were analyzed in 96-well plates by observing the conversions of the linked reactions of UDP + phosphoenolpyruvate to UTP + pyruvate by pyruvate kinase, followed by pyruvate + NADH to lactate + NAD<sup>+</sup> by lactate dehydrogenase spectrophotometrically at 340 nm every minute for 10 minutes.<sup>28</sup> Cytosolic and microsomal proteins were measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method.

### *Statistical analysis*

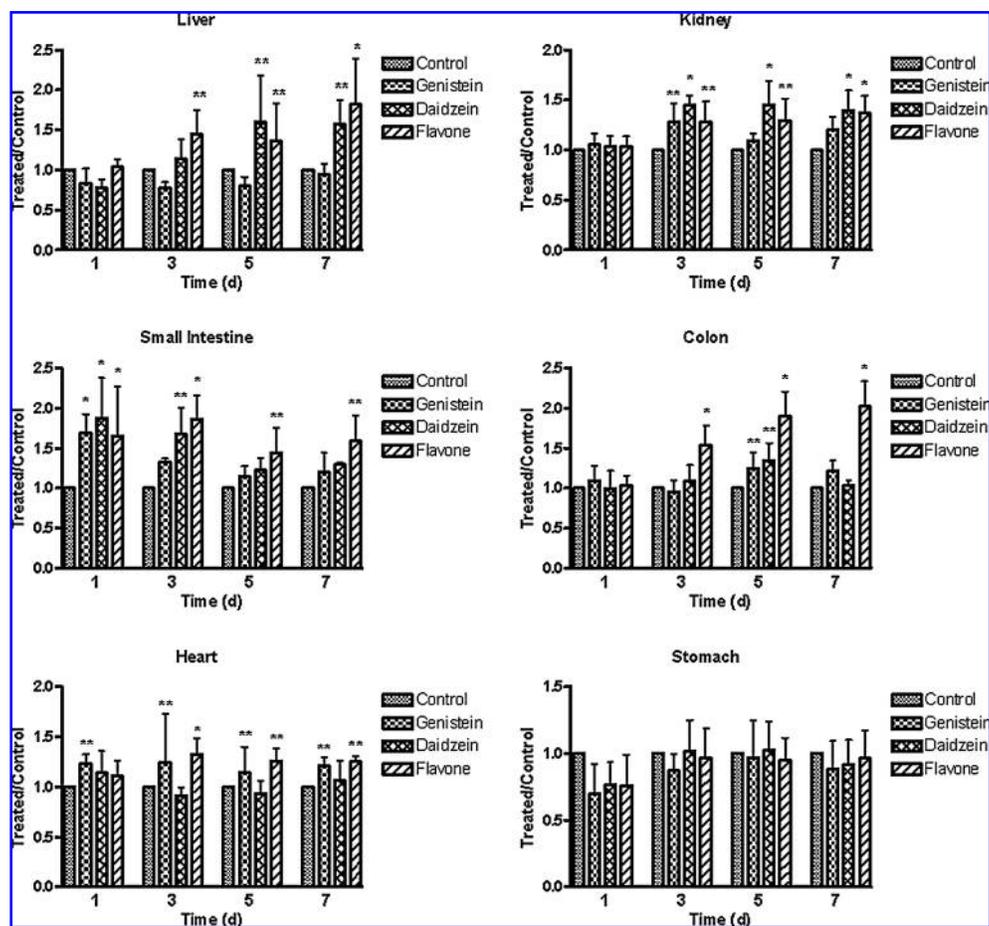
All data were analyzed using the software from SigmaStat (Point Richmond, CA, USA). Data were analyzed using analysis of variance, followed by the Student-Newman-Keuls test for all pairwise comparisons of means to detect differences compared to controls. An  $\alpha$  level of 0.05 was used to determine significance.

## RESULTS

### *Body and liver weights*

The initial and final body weights for each treatment group did not differ compared to the control group. The

**FIG. 1.** QR activity in male Swiss Webster mice reflects isoflavone- and tissue-specific responses. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by the Student-Newman-Keuls test.



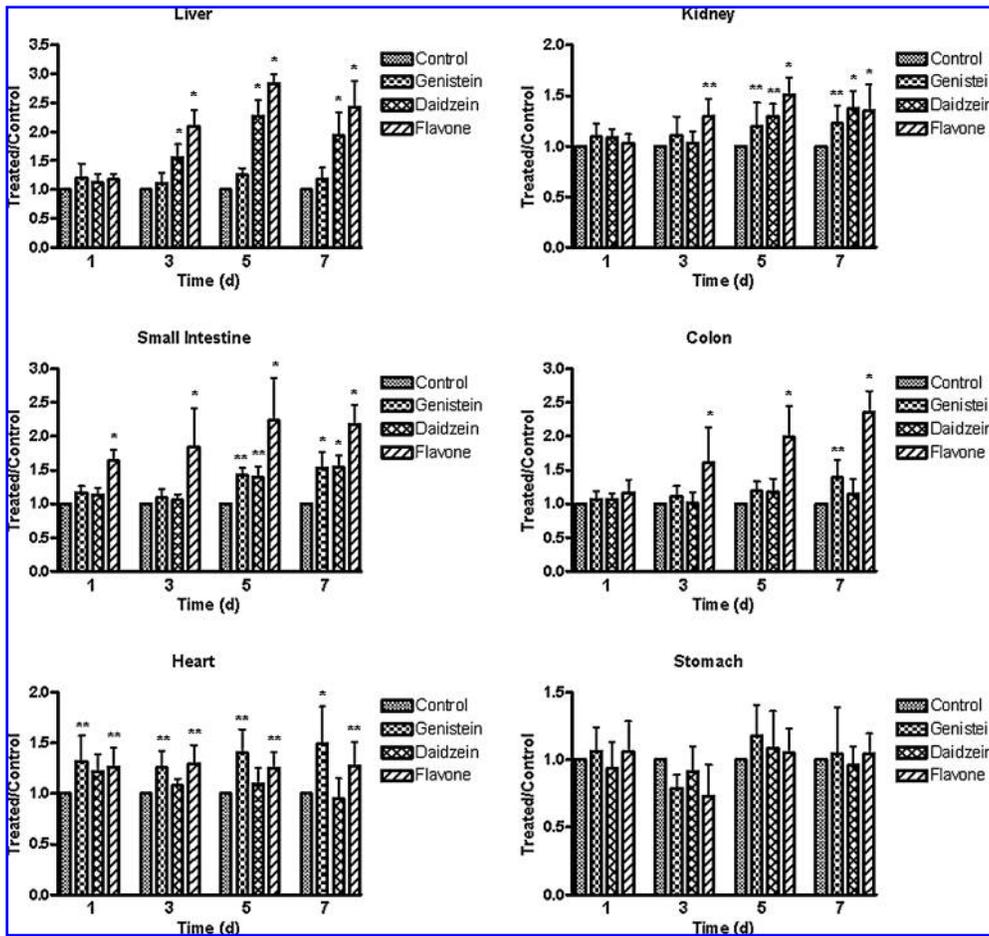
average initial and final body weights for males were 21.6 g and 28.6 g, respectively; those for females were 28.2 g and 32.9 g, respectively. Liver/body weight ratios for the treatment groups were not different compared to the control group (data not shown).

#### *Genistein and daidzein increase QR activity in various tissues*

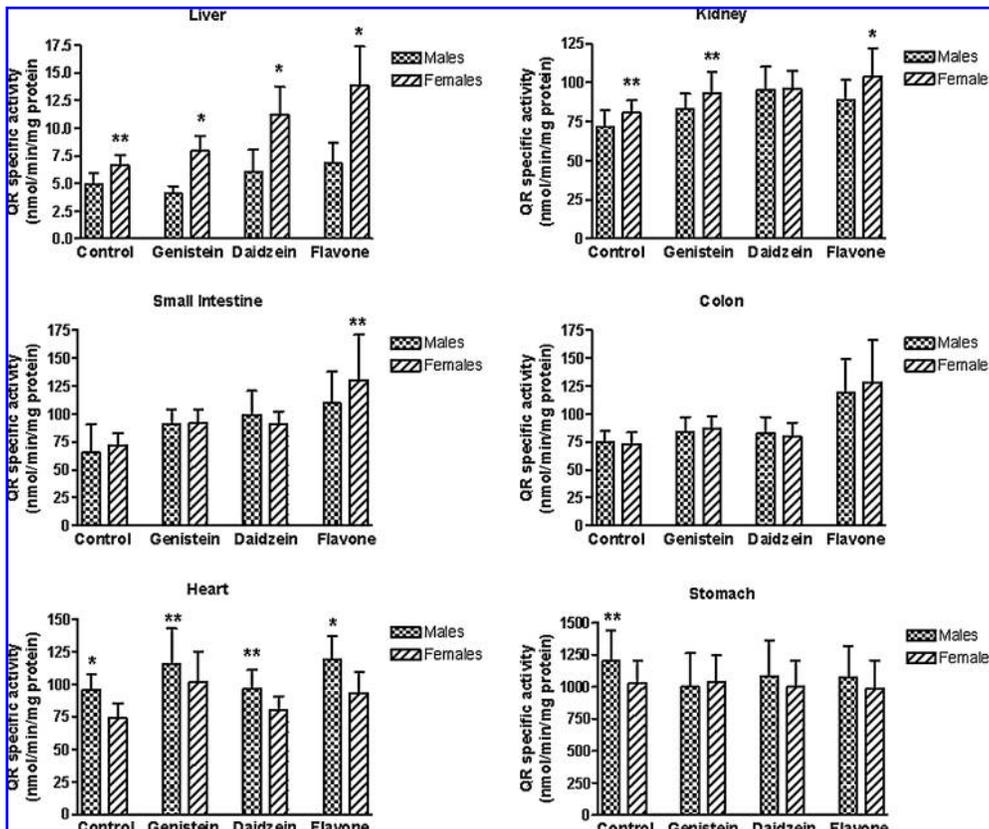
In the male mice (Fig. 1), daidzein increased liver QR activity at days 5 and 7 by 1.6-fold ( $P < .05$ ) (average liver QR specific activity for the control mice was 4.9 nmol/minute/mg of protein). In contrast, there were no significant differences found in liver QR activity between the genistein-treated and the control mice. Kidney QR activity was increased by daidzein at days 3, 5, and 7 by 1.4–1.5-fold ( $P < .001$ ) and by genistein at day 3 by 1.3-fold ( $P < .05$ ) compared to the control mice (average kidney QR specific activity for the control mice was 71.95 nmol/minute/mg of protein). The activity of QR in the small intestine was increased by genistein at day 1 by 1.7-fold ( $P < .001$ ) and by daidzein at days 1 and 3 by 1.7–1.9-fold ( $P < .05$ –.001) compared to the controls (average small intestine QR specific activity for the control mice was 69.13 nmol/minute/mg of protein). Genistein and daidzein had little impact on

colon QR activity (average colon QR specific activity for the control mice was 74.34 nmol/minute/mg of protein). The activity of QR in the heart was increased by genistein (but not daidzein) treatment at all time points by 1.2–1.3-fold ( $P < .05$ ) (average heart QR specific activity for the control mice was 95.71 nmol/minute/mg of protein). QR activity in the stomach was not significantly different from control mice for any of the phytochemical treatment groups (average stomach QR specific activity for the control mice was 1,203 nmol/minute/mg of protein). Flavone significantly increased QR activity (at various time points) for all tissues, except the stomach.

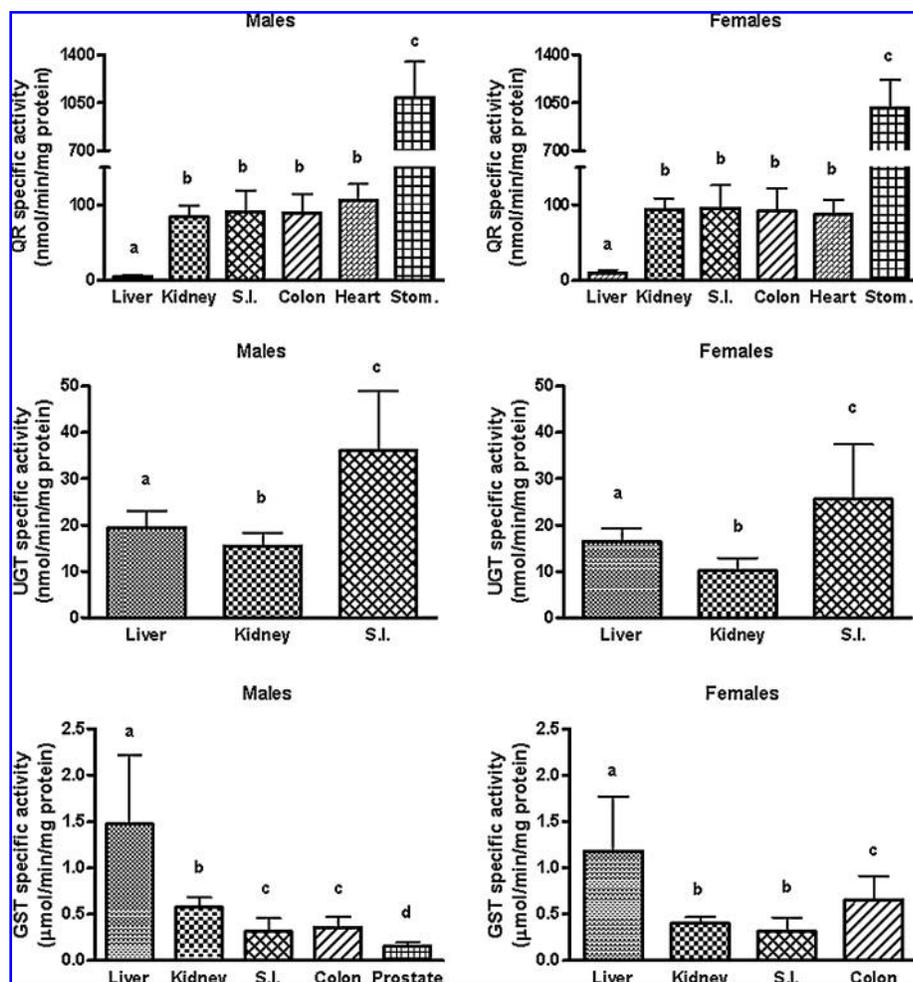
In the female mice (Fig. 2), daidzein increased liver QR activity at days 3, 5, and 7 by 1.6–2.3-fold ( $P < .001$ ) compared to the controls (average liver QR specific activity for the control mice was 6.67 nmol/minute/mg of protein). In contrast, there were no significant differences found in liver QR activity between the genistein-treated and the control mice. Kidney QR activity was increased by daidzein at days 5 and 7 by 1.3–1.4-fold ( $P < .05$ –.001) and by genistein at days 5 and 7 by 1.3-fold ( $P < .05$ ) compared to the controls (average kidney QR specific activity for the control mice was 80.73 nmol/minute/mg of protein). Genistein and daidzein treatments increased small intestine QR activity at days 5 and 7 by 1.4–1.5-fold ( $P < .05$ –.001)



**FIG. 2.** QR activity in female Swiss Webster mice reflects isoflavone- and tissue-specific responses. The mice were fed control diet (casein AIN-93G) or diet control supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by the Student-Newman-Keuls test.



**FIG. 3.** Sex comparisons of QR specific activity in various tissues. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet for 1, 3, 5, or 7 days. Data are mean  $\pm$  SD values from 20 mice per group (includes five mice per time point). \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by the Student-Newman-Keuls test.



**FIG. 4.** Tissue comparisons for QR, UGT, and GST specific activity for Swiss Webster mice. The mice were fed control diet (casein AIN-93G) or control diet supplemented with genistein, daidzein, or flavone at 1,500 mg/kg of diet for 1, 3, 5, or 7 days. Data are mean  $\pm$  SD values from 80 mice per group (includes all days and treatments). Groups not sharing the same letter differ significantly ( $P < .001$ );  $P < .05$  and analysis of variance were used, followed by the Student-Newman-Keuls test.

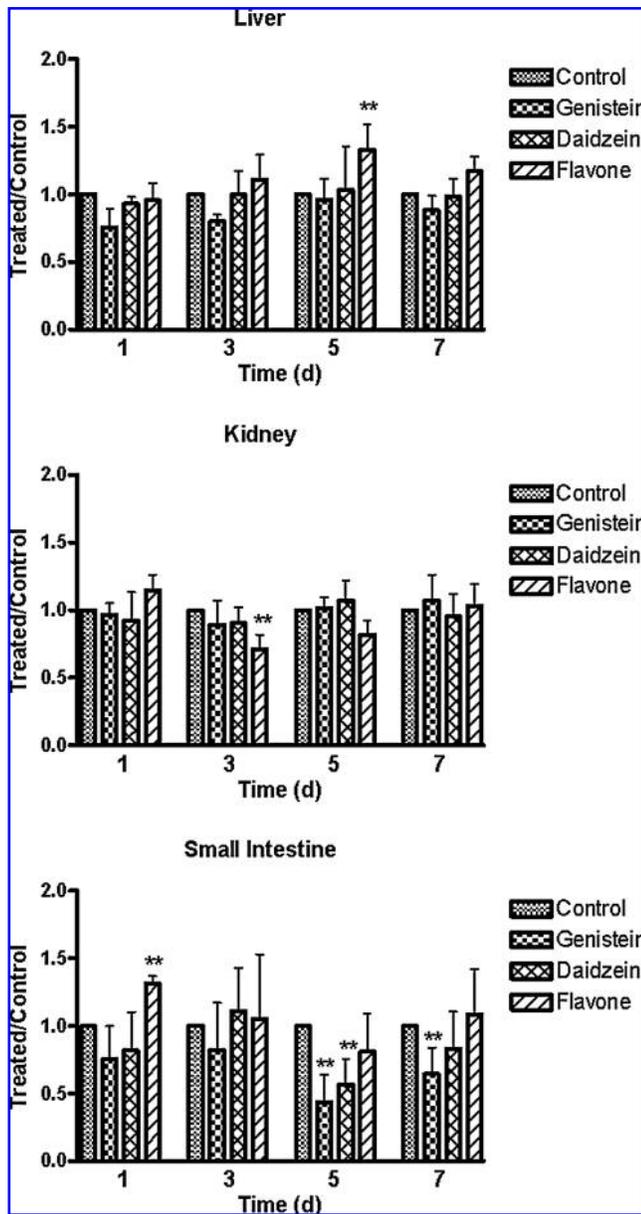
compared to the control mice (average small intestine QR specific activity for the control mice was 71.53 nmol/minute/mg of protein). Genistein and daidzein had little impact on colon QR activity (average colon QR specific activity for the control mice was 73.01 nmol/minute/mg of protein). The activity of QR in the heart was increased by genistein (but not daidzein) treatment at all time points by 1.3–1.5-fold ( $P < .05$ –.001) (average heart QR specific activity for the control mice was 74.07 nmol/minute/mg of protein). QR activity in the stomach was not significantly different from control mice for any of the phytochemical treatment groups (average stomach QR specific activity for the control mice was 1,030.89 nmol/minute/mg of protein). Flavone significantly increased QR activity (at various time points) for all tissues, except the stomach.

For the sex effects (Fig. 3), the females had higher liver (all treatments) and kidney (except daidzein treatment) QR activity compared to the males. There were no significant sex differences (except for flavone treatment) in small intestine QR activity. There were no significant sex differences between treatment groups for colon QR activity. The males had higher heart QR activity for all treatments compared to the females. There were no significant sex differences between groups except for the control mice where

the males had higher stomach QR activities compared to the females. Across tissues (Fig. 4), the liver was the lowest, and the stomach was the highest for both sexes, in QR activity.

#### *Genistein and daidzein decrease UGT activity in male and female mice*

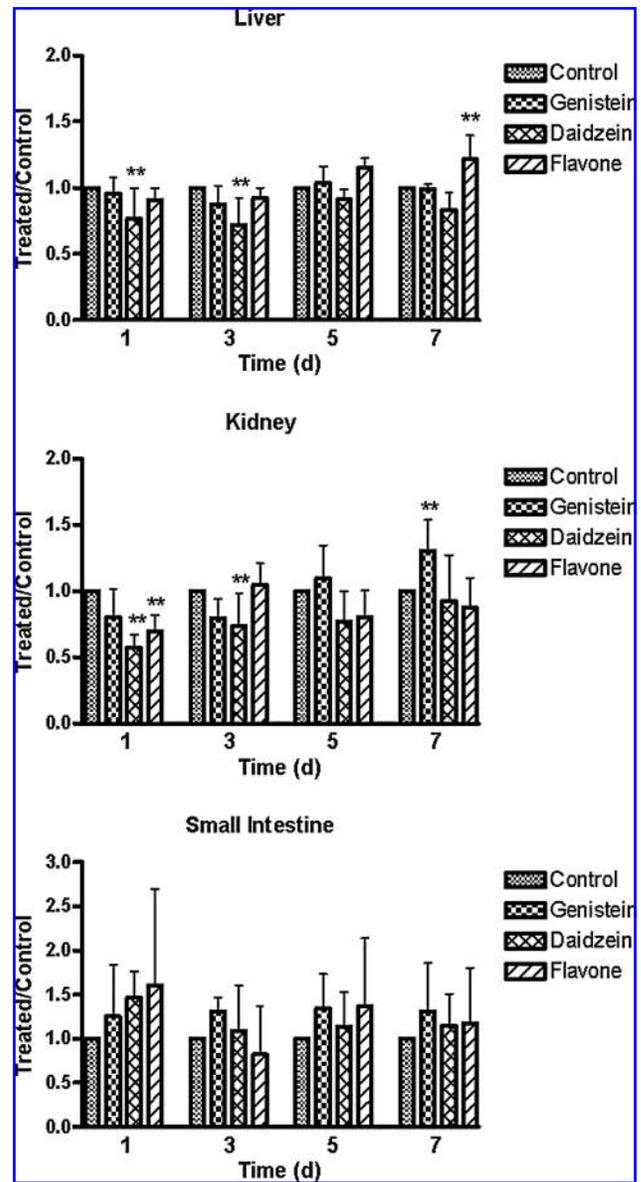
Genistein, at days 5 and 7 ( $P < .05$ ), and daidzein, at day 5 ( $P < .05$ ), decreased small intestine UGT activity in the males compared to the control mice (Fig. 5). There were no significant differences observed in the liver and kidney activities for genistein- and daidzein-treated male mice. In contrast to the males, daidzein decreased liver and kidney UGT activities in the female mice at days 1 and 3 ( $P < .05$ ) (Fig. 6). There were no significant differences observed in the small intestine for genistein- and daidzein-treated female mice. In general, flavone failed to induce UGT activity in both sexes. Regarding the sex differences (Fig. 7), the males had higher liver (except genistein), kidney (all treatments), and small intestine (except genistein) UGT activities compared to the females. Across the examined tissues, the scale of UGT activity for both sexes was the small intestine  $>$  liver  $>$  kidney (Fig. 4).



**FIG. 5.** UGT activity in male Swiss Webster mice. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

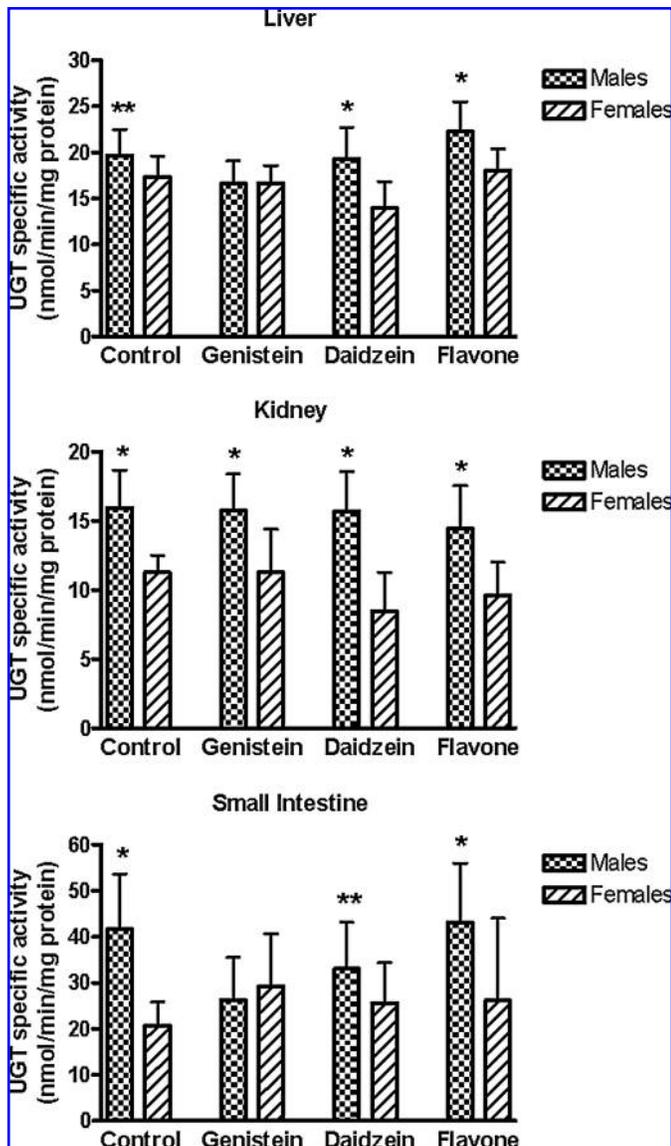
*Genistein and daidzein modulate GST activity in female mice*

In general, there were no significant differences in GST activity for the males treated with genistein or daidzein in the examined tissues (Fig. 8). In the females, liver GST activity was decreased by genistein at all time points ( $P < .001$ ) compared to the control mice (Fig. 9). In contrast, genistein and daidzein increased kidney GST activity at days 5 and 7 ( $P < .05$ ) in the females. There were up and



**FIG. 6.** UGT activity in female Swiss Webster mice. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

down modulations by genistein and daidzein in the small intestine and colon for the female mice. In both sexes, flavone increased GST activity in all tissues (except for the colon in the males) at various days compared to the control mice. For the sex effects, there was a significant difference in liver GST activity as the males had higher values compared to the females for the flavone- and genistein-treated mice (Fig. 10). The males also had higher kidney (all treatments), but lower colon (all treatments), GST activity compared to the females. Across tissues, the scale of GST



**FIG. 7.** Sex comparisons of UGT specific activity. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet for 1, 3, 5, or 7 days. Data are mean  $\pm$  SD values from 20 mice per group (includes five mice per time point). \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

activity in the male mice was the liver > kidney > small intestine = colon > prostate; the female scale was the liver > colon > kidney = small intestine (Fig. 4).

## DISCUSSION

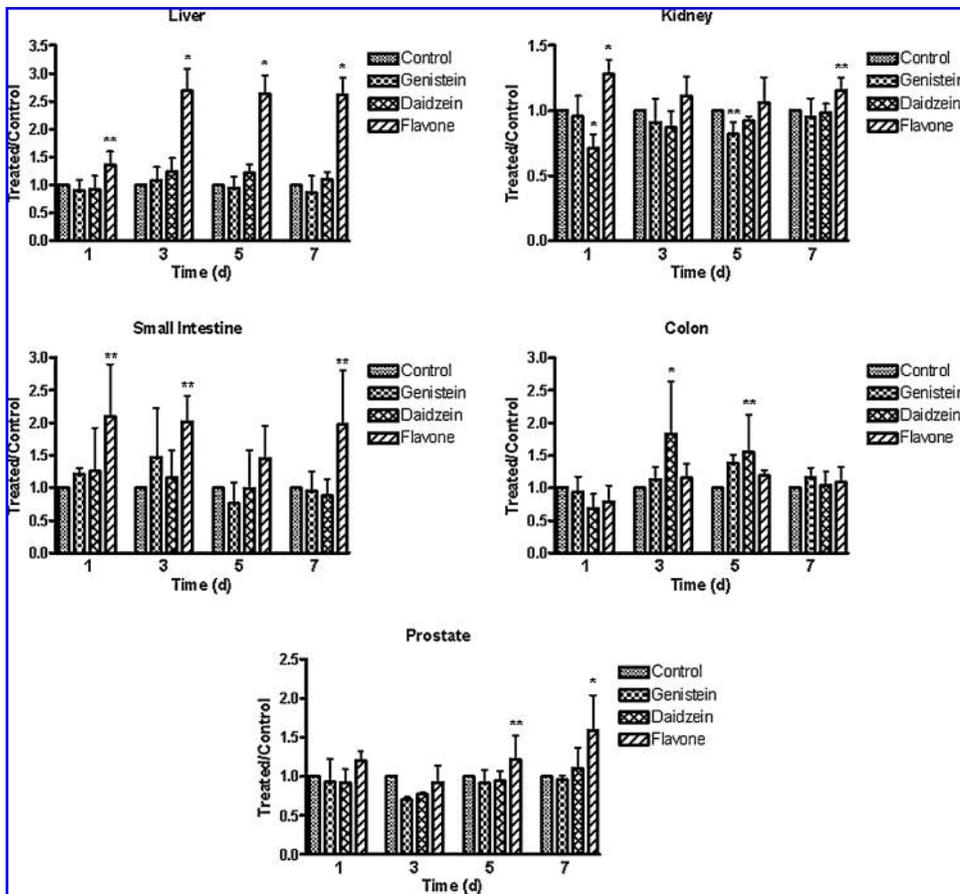
This study provides insights into the actions of isolated isoflavones in mice and demonstrates that isoflavone-specific responses in basal activities of xenobiotic metabolizing enzymes can occur after the short-term feeding of bioactive soy phytochemicals. Genistein and daidzein increased QR activity in the kidney, having similar increases

in enzyme activity in both sexes over multiple time points. These results coincide with another study that demonstrated an increase in colon and kidney QR activities in rats treated with soy protein (isoflavones at 810 mg/kg of diet) for 2 and 13 weeks.<sup>21</sup> The isoflavones in this study had fewer effects in the colon and none in the stomach. However, in both sexes, genistein and daidzein increased QR activity in the small intestine, and genistein increased QR activity in the heart. In liver tissue, only daidzein induced QR activity. Another investigation,<sup>19</sup> using the same methodology to measure QR activity as this study, resulted in both genistein and daidzein inducing QR in murine hepatoma cells. The increase in QR activity was in a bifunctional manner, involving an arylhydrocarbon receptor-dependent mechanism, as well as weakly in a monofunctional mode independent of the arylhydrocarbon receptor in mutant cells. The differing genistein results could be due to the use of cells versus whole animal model (used in this study), which includes various isoflavone metabolites possibly affecting QR activity.

Higher liver QR activity in female mice may be explained by combined effects of daidzein binding to the ER in addition to  $17\beta$ -estradiol.<sup>15</sup> ER $\beta$  has been demonstrated to bind to the QR antioxidant response element, increasing QR mRNA and protein.<sup>29</sup> QR specific activity was the lowest in liver tissue and highest in the stomach. Similar results have been reported elsewhere demonstrating the liver QR activity is low compared to the stomach, small intestine, colon, and kidney, with the stomach being the highest. It is suggested that these tissues are evolutionarily adapted in response to exposure of high concentrations of toxic, mutagenic, and carcinogenic substances.<sup>30</sup>

Examination of UGT activity revealed limited effects of isoflavones, with few significant differences observed compared to control mice. These results are comparable with an observation that genistein did not affect hepatic UGT activity in female rats.<sup>31</sup> In contrast, it has been demonstrated in previous reports that soy isoflavones can increase UGT activity. The general lack of UGT activity induction by phytochemicals seen in this study may be due to study design aspects that differ from the existing literature, such as the use of isolated isoflavones versus soy protein,<sup>21</sup> the length of feeding time,<sup>21,22,32</sup> the use of whole animal as opposed to cells,<sup>33</sup> differing specificity of model substrates such as observing phenol-UGT versus testosterone-UGT activity<sup>33</sup> or other substrates, and using basal constitutive enzyme activation rather than a carcinogen after isoflavone treatment.<sup>23</sup> Finally, the inducing effect of UGT1A1 has been proposed to be arylhydrocarbon receptor independent,<sup>34</sup> which would explain why the positive control flavone, an arylhydrocarbon receptor ligand, did not induce UGT activity.

In general, the males had higher UGT activity in the liver, kidney, and small intestine. Higher concentrations of ER $\alpha$  can precipitate nuclear factor erythroid 2 p45-related factor 2, inhibiting it from translocating into the nucleus and binding to the antioxidant response element, resulting in decreased transcription of the *UGT* gene in female mice,<sup>35-37</sup> consistent with low UGT activity as we observed. The small



**FIG. 8.** GST activity in male Swiss Webster mice. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

intestine is the most important organ for glucuronidation,<sup>38</sup> as evidenced by its higher UGT activity compared to the liver and kidney in the present study.

The effects of isoflavones on GST activity were variable with respect to gender, isoflavone type, and tissue, with the largest significant difference from the control being a decrease of hepatic GST activity in females fed genistein. These findings are consistent with the variable effects that have been previously reported. For example, genistein repressed GST Ya antioxidant response element-dependent gene expression in COS I cells expressing ER $\alpha$  and ER $\beta$ .<sup>39</sup> In contrast, soy protein has been demonstrated to increase total GST activity in one study with rats,<sup>21</sup> whereas most other studies have shown no effect of genistein on GST activity in mouse cells,<sup>40</sup> in mice fed soya bean flakes for 90 days,<sup>22</sup> and in rats.<sup>23</sup> Isoflavones binding to ER $\beta$  (and ER $\beta$  subsequently binding to the antioxidant response element) are not likely to have a significant impact on total GST expression, because of the many GST isozymes, only GST  $\pi$  appears to be regulated by ER $\beta$ .<sup>41</sup> Males have been reported to have higher amounts of liver and kidney GST isozymes than females,<sup>42</sup> a finding in agreement with the present study with the exception of the colon. The 1-chloro-2,4-dinitrobenzene assay used in this study does not detect every isozyme, but collectively measures  $\alpha$ ,  $\mu$ , and  $\pi$  GST; thus our methodology does not allow for conclusions re-

garding isozyme-specific tissue responses to isoflavones. Flavone increased GST activity in a variety of tissues for both sexes, consistent with a previous report where flavone induced GST activity.<sup>32</sup>

Equal results from the intestinal bacterial metabolism of daidzein and might be responsible for some of the effects seen with daidzein. Approximately 50% of equol circulates as the unbound form, so its potency and receptor binding are much higher than those of estrogens.<sup>43</sup> Equol has been demonstrated to bind to ER $\alpha$  and ER $\beta$  similarly to genistein,<sup>15,43</sup> which has an approximately 30-fold higher binding affinity for ER $\beta$  compared to ER $\alpha$ .<sup>43</sup> Only 30–50% of humans are equol producers, whereas all rodents produce equol.<sup>43</sup> Therefore, this might account for some of the differing results seen in human,<sup>44</sup> cell culture,<sup>18,19</sup> and animal<sup>21,43,45</sup> studies, with respect to daidzein.

High amounts of isoflavones are suggested to be needed in rodents in order to be comparable in humans as PII modulators, for example, feeding rodents levels of isoflavones two to three times higher than the usual human soy-based diet.<sup>21,46</sup> It is difficult to directly extrapolate a rodent's intake of isoflavone to a human's intake, because of their differing metabolism profiles. However, the experimental diet composition used in the present study was based on intakes and blood concentrations of isoflavones achieved in rodents that are comparable to typical Asian population

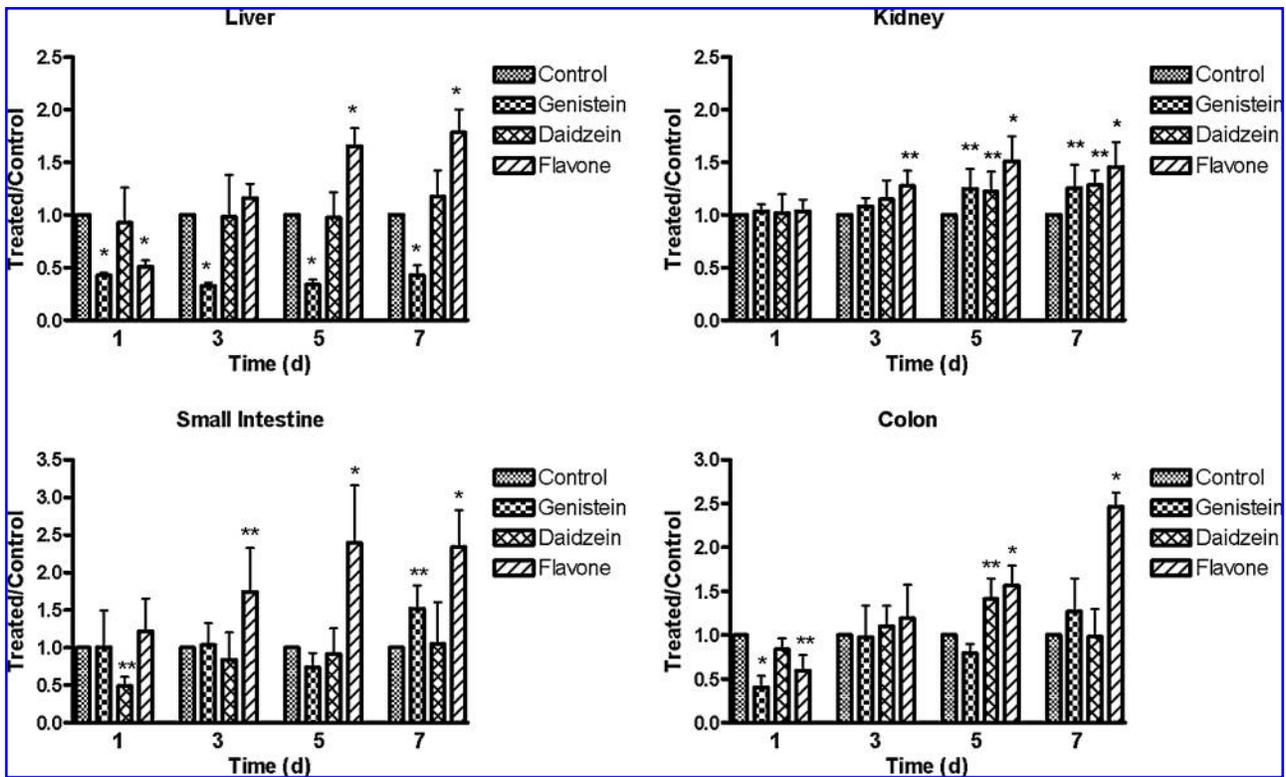


FIG. 9. GST activity in female Swiss Webster mice. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet. Data are mean  $\pm$  SD values from five mice per group, expressed as treated/control ratios. \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

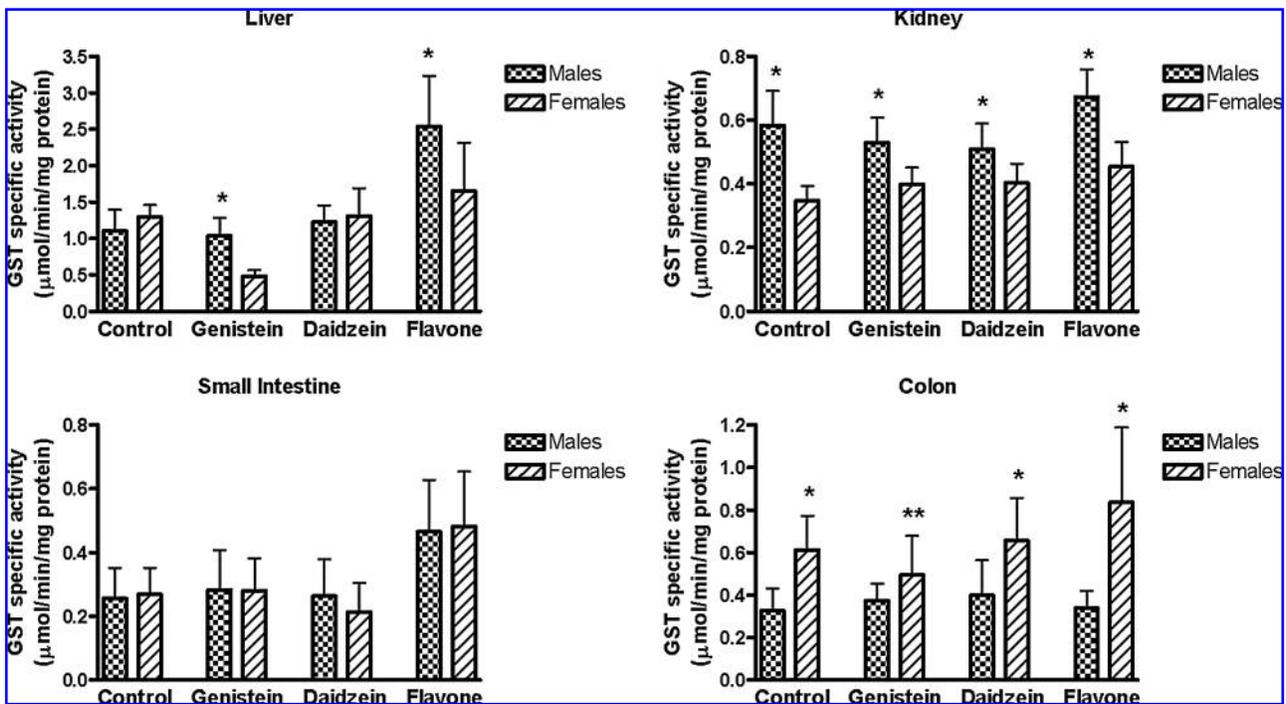


FIG. 10. Sex comparisons of GST specific activity. The mice were fed control diet (casein AIN-93G) or control diet supplemented with phytochemical at 1,500 mg/kg of diet for 1, 3, 5, or 7 days. Data are mean  $\pm$  SD values from 20 mice per group (includes five mice per time point). \*\* $P < .05$ , \* $P < .001$ , significant difference compared to control;  $P < .05$  and analysis of variance were used, followed by Student-Newman-Keuls test.

blood concentrations in high isoflavone-consuming areas. Previous investigators have fed mice genistein at 500 mg/kg of diet,<sup>47</sup> genistein, daidzein, and equol at 1,000 mg/kg of diet,<sup>48</sup> genistein at 1,500 mg/kg of diet,<sup>3</sup> and isoflavones (genistein, daidzein, and glycitein mix) at 1,750 mg/kg of diet.<sup>11</sup> The aforementioned studies found the serum isoflavone concentrations (0.4–3.8  $\mu$ M) following soy aglycone feeding were comparable to those found in humans consuming a soy-rich meal, with genistein peaking at up to 5  $\mu$ M. Rodents metabolize genistein at a faster rate than humans; thus, genistein must be administered at a higher level to obtain equivalent human serum concentrations.<sup>3</sup> Therefore, this current study used similar high physiologic concentrations of genistein and daidzein (1,500 mg/kg of diet) to determine if they modulate phase I and PII metabolism. A drawback of the study is that plasma isoflavone concentrations were not measured, because of technical limitations. As indicated by the weight gain and liver/body weight ratios observed in the current study, 1,500 mg/kg dietary genistein and daidzein did not result in any overt toxic effects. Consistent with our findings, mice and rats fed very high concentrations of genistein (20 and 2,000 mg/kg of body weight, respectively) did not exhibit any adverse effects in other studies.<sup>49</sup>

In conclusion, our results support the hypothesis that there are isoflavone-specific, time-, and sex-related effects resulting in the modulation of PII enzymes in a variety of tissues in a rodent model under short-term feeding and basal conditions. QR activity was induced by isoflavones in the liver, kidney, small intestine, and heart, whereas genistein inhibited liver GST activity in the females. In contrast, both genistein and daidzein increased kidney GST activity in the female mice. In general, the greatest effects of isoflavones on PII enzymes were observed in liver and kidney tissues, occurring at day 3 and peaking by day 5. Sex effects in the liver and kidney included the females exhibiting higher QR activities and the males exhibiting higher UGT and GST activities. Distribution of xenobiotic enzymes and differing isozyme profiles can contribute to tissue-specific responses to plant-derived bioactive compounds as seen in our study and other literature reports; however, specific isozymes were not assessed in this study. The current results extend previous knowledge regarding effects of high physiologic concentrations of dietary aglycone isoflavones on biotransformation enzymes by demonstrating modulation of typical constitutive enzyme levels. This has implications for metabolism of the phytochemicals themselves in addition to chemoprevention against carcinogenic dietary and environmental compounds. Further studies are needed to add insight into the cellular and molecular mechanisms of PII modulation by isoflavones, their metabolites, and other bioactive soy phytochemicals.

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#### AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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