Daidzein and Genistein Are Converted to Equol and 5-Hydroxy-Equol by Human Intestinal *Slackia isoflavoniconvertens* in Gnotobiotic Rats\(^1\)\(^-\)\(^3\)

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**Abstract**

Intestinal conversion of the isoflavone daidzein to the bioactive equol is exclusively catalyzed by gut bacteria, but a direct role in equol formation under in vivo conditions has not yet been demonstrated. *Slackia isoflavoniconvertens* is one of the few equol-forming gut bacteria isolated from humans and, moreover, it also converts genistein to 5-hydroxy-equol. To demonstrate the isoflavone-converting ability of *S. isoflavoniconvertens* in vivo, the metabolism of dietary daidzein and genistein was investigated in male and female rats harboring a simplified human microbiota without (control) or with *S. isoflavoniconvertens* (SIA). Feces, urine, intestinal contents, and plasma of the rats were analyzed for daidzein, genistein, and their metabolites. Equol and 5-hydroxy-equol were found in intestinal contents, feces, and urine of SIA rats but not in the corresponding samples of the control rats. 5-Hydroxy-equol was present at much lower concentrations than equol and the main metabolite produced from genistein was the intermediate dihydrogenistein. The plasma of SIA rats contained equol but no 5-hydroxy-equol. Equol formation had no effect on plasma concentrations of the insulin-like growth factor I. The concentrations of daidzein and genistein were considerably lower in all samples of the SIA rats than in those of the control rats. Male SIA rats had higher intestinal and fecal concentrations of the isoflavones and their metabolites than female SIA rats. The observed activity in the rat model indicates that *S. isoflavoniconvertens* is capable of contributing in vivo to the bioactivation of daidzein and genistein by formation of equol and 5-hydroxy-equol. J. Nutr. 142: 40–46, 2012.

**Introduction**

Isoflavones are polyphenolic secondary plant metabolites that have been implicated in the prevention of breast, prostate, and colon cancer, osteoporosis, cardiovascular disease, and obesity (1–4). The protective effects of dietary isoflavones may be due to their selective binding to the estrogen receptors and estrogen-related receptors or inhibition of enzymes (2,5,6). Isoflavones also have antioxidative properties (3) and they may target the factors promoting their colonization in the human gut are still unknown (10). A few human intestinal bacteria catalyzing the bioactivation of daidzein via dihydrodaidzein to equol have been identified: *Adlercreutzia equolifaciens*, *Slackia isoflavoniconvertens*, *Slackia equolifaciens*, and *Lactococcus garvieae* (11–14). The activity of a specific equol-forming bacterium under in vivo conditions has not been demonstrated yet. In a previous study, the ability to form equol was solely transferred to germfree rats by association with the complete fecal microbiota from a high equol-producing human donor. These rats excreted equol in their urine, whereas rats associated with the fecal microbiota of a donor low in equol production excreted no equol (15). The corresponding pathway of genistein conversion via dihydrogenistein to 5-hydroxy-equol was so far been reported for the human intestinal *S. isoflavoniconvertens* (13) and *S. equolifaciens* (11) and for *Enterorhabdus mucosicola* isolated from the

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3 Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
4 Abbreviations used: BW, body weight; IGF, insulin-like growth factor; SIA, *Slackia isoflavoniconvertens*-associated; SIHUMIx, simplified human microbiota.
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mouse gut (16,17). However, the formation of 5-hydroxy-equol in vivo has not yet been observed in either animal or human studies.

The objective of the present study was to demonstrate the ability of S. isoflavoniconvertens to bioactivate daidzein to equol and genistein to 5-hydroxy-equol in vivo. For this purpose, the formation of bacterial metabolites derived from daidzein and genistein was analyzed in rats colonized with a simplified human microbiota with or without S. isoflavoniconvertens. Moreover, the influence of equol formation on plasma IGF-I was studied.

**Materials and Methods**

**Chemicals.** Daidzein and genistein were purchased from Roth. Dihydrodaidzein was obtained from Toronto Research Chemicals. Equol was purchased from Fluka. Dihydrogenistin and 3-hydroxy-equol were available from a previous study (16).

**Rats and treatment.** The SIHUMix rat model associated with eight intestinal bacteria was previously established in germfree Sprague Dawley rats bred in the animal facility of the German Institute of Human Nutrition Potsdam-Rehbruecke from stock obtained from Charles River (18). The SIHUMix rats were housed as previously described (18) and had unrestricted access to autoclaved drinking water and an irradiated standard rodent diet (Altromin 1310 fortified, per kg: 225 g of crude protein, 50 g of crude fat, 45 g of crude fiber, 65 g of crude ash, 505 g of nitrogen-free extracts).

Three 12-wk-old male SIHUMix rats were associated with S. isoflavoniconvertens DSM 22006 (13) via i.g. application of a bacterial suspension (500 μL, ~2.2 × 10^7 cells) on three successive days. A 100-mL overnight culture of S. isoflavoniconvertens grown in brain heart infusion broth (gas phase, H2:CO2, 80:20, v:v) was centrifuged at 9000 g for 15 min at 4°C and the cell pellet was resuspended in 5 mL of reduced PBS (per L: 8.5 g of NaCl, 0.3 g of KH2PO4, 0.6 g of Na2HPO4, 0.25 g of cysteine/HCl × H2O, 0.1 g of Bacto peptone, 0.001 g of resazurin; pH 7). The establishment of S. isoflavoniconvertens in the gut of the SIHUMix rats was monitored by fluorescent in situ hybridization and by analyzing feces for the presence of equol. The male SIA rats were mated to female SIHUMix rats resulting in SIA offspring, which were used in this study and compared to their SIHUMix counterparts as controls. The rats were kept as described above. Examination of the diet by acid hydrolysis and liquid-liquid extraction as reported elsewhere (19) followed by HPLC analysis revealed total amounts of 1310 nmol daidzein and 1360 nmol genistein/g wet weight. The rats consumed 0.128 ± 0.028 g diet/g BW • d). Ten 8- to 10-wk-old SIA rats (5 males, BW 359 ± 25 g; 5 females, BW 258 ± 16 g) and ten 8- to 10-wk-old control rats (5 males, BW 321 ± 20 g; 5 females, BW 229 ± 17 g) were placed in metabolic cages (Techniplast) and urine and feces were collected for 12 h. The rats were subsequently killed by decapitation and blood was collected in heparinized tubes (Sarstedt). In addition, contents of small intestine, cecum, and colon were collected. The Ministry of Environment, Health and Consumer Protection of the Federal State of Brandenburg, Germany approved the protocol of the animal experiments according to §8.1 Animal Welfare Act (approval no. 23-2347-8-16-2008).

**Test of the SIHUMix consortium for isoflavone conversion.** Freshly collected fecal samples of SIHUMix rats were 10-fold diluted (wt/v) with reduced PBS and 100-μL aliquots were incubated with daidzein and genistein and tested for their ability to convert these isoflavones as previously described (16). Following the incubation of the fecal suspensions with daidzein or genistein for 28 h under strict anoxic conditions, 95% of the added daidzein and 86% of the genistein were still present and none of the typical degradation products were detected. This indicated that the SIHUMix consortium converted neither daidzein nor genistein in vitro. Incubation of daidzein and genistein without bacteria resulted in a recovery of 112 and 99%, respectively.

**Enumeration of bacteria.** Numbers of total intestinal bacteria and of S. isoflavoniconvertens in suspensions of feces or gut contents were determined by microscopy-based manual counting after paraformaldehyde/ethanol fixation and fluorescent in situ hybridization as previously described (20). Total bacteria were detected by a mixture of 5 16S rRNA-targeted oligonucleotide probes (EubMix) (20). For quantification of S. isoflavoniconvertens, a 16S rRNA-targeted oligonucleotide probe (S. is0595, 5’-Cy3-GCATCCAGGGCTCTCCGTT) was used. This probe is based on a species-specific primer sequence evaluated for quantitative RT-PCR (21). Its specificity for S. isoflavoniconvertens within the organisms of the SIHUMix consortium was demonstrated in vitro and in vivo. The hybridization temperature of the S.iso0595 probe was 46°C.

**Sample preparation.** Blood was centrifuged at 1000 × g for 15 min at 4°C and plasma collected. Urine was centrifuged at 10,000 × g for 5 min at 4°C and the supernatant was sterile filtered and acidified with ascorbic acid (final concentration, 1%). Two volumes of ethyl acetate were added to aliquots of plasma (200 μL), urine (500 μL), and suspensions of feces and gut contents (275 mg in 500 μL of water and sonicated for 10 min), shaken for 10 min at room temperature, and centrifuged at 9000 × g (plasma, 14,000 × g) for 5 min at 4°C. The extraction steps were repeated 3 times. The pooled supernatants were dried by vacuum centrifugation. The residues were dissolved in 200 μL (plasma, 80 μL) of 70% (v:v) aqueous methanol and centrifuged at 14,000 × g for 5 min at room temperature. The supernatants were analyzed by HPLC. All extractions were performed in duplicate. To include conjugated metabolites, samples were incubated with β-glucuronidase/sulfatase before extraction as previously described (22).

The recovery of the compounds was determined by spiking plasma, urine, and fecal samples of germfree Sprague Dawley rats fed a semipurified, flavonoid-free diet (22) with defined amounts of daidzein, genistein, dihydrodaidzein, dihydrogenistin, and equol followed by extraction as described above (Supplemental Table 1). The concentrations determined by HPLC analyses were accordingly corrected. Fecal recoveries were used for correction of concentrations in intestinal contents.

**HPLC analysis.** Samples were analyzed by a HPLC system previously described (22). The column temperature was maintained at 37°C. The injection volume was 20 μL. The solvent was a mixture of (A) water: acetic acid (98:2, v:v) and (B) methanol and delivered at 1 mL/min in a gradient mode (10–55% B in 35 min, 55% B for 3 min, 55–100% B in 1 min). Detection was at 280 nm. For control of the HPLC system and data processing, the Chromeleon software version 6.40 ( Dionex) was used. Calibration curves were used for quantification. Owing to its limited availability, 5-hydroxy-equol was quantified based on the calibration curve of equol. The detection limits were 0.1 μmol/L for daidzein, genistein, and dihydrodaidzein, 0.5 μmol/L for dihydrogenistin, and 1.0 μmol/L for equol.

**Plasma IGF-I.** Plasma samples were assayed for IGF-I concentration by using the Quantikine Mouse/Rat IGF-I Immunoassay (R&D Systems) according to the manufacturer’s instructions.

**Calculations and statistics.** The treatment of samples with β-glucuronidase/sulfatase provided the total concentrations of each isoflavone or isoflavone metabolite. These values were given unless otherwise specified. The concentration of conjugates was calculated each as the difference of the total concentration and the concentration of the free compound.

Statistical analyses were conducted using SPSS 19.0 (IBM). Values were tested for normal distribution with the Kolmogorov-Smirnov test. For normally distributed data (bacterial cell counts), differences were checked for significance using the unpaired t test or repeated-measures ANOVA with a post hoc paired t test. Differences of non-normally distributed data (concentrations of isoflavones, isoflavone metabolites, IGF-I) were analyzed using the Mann-Whitney U test for independent samples and the Wilcoxon test for dependent samples. The following comparisons were made: male SIA rats vs. male control rats, female SIA rats vs. female control rats, SIA rats vs. control rats, gut contents and feces to one another, and isoflavones and their metabolites to one another. Values are means ± SD or medians (range). Differences of P ≤ 0.05 were considered significant.

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Results

Establishment of *S. isoflavoniconvertens* in the rats. *S. isoflavoniconvertens* stably colonized the intestine of the SIHUMIx rats and was also transferred to the offspring. In the 8- to 10-wk-old SIA rats, *S. isoflavoniconvertens* occurred in considerable numbers in small intestinal, cecal, and colonic contents (Table 1). The counts of total bacteria in the small intestinal contents of SIA and control rats and of *S. isoflavoniconvertens* in SIA rats were lower by 3 orders of magnitude than those in the cecal or colonic contents (Table 1). *S. isoflavoniconvertens* accounted for 2.9% of total bacterial cells in the small intestine, 1.5% in the cecum, and 2.5% in the colon of SIA rats. Gender-dependent differences were not observed.

**Daidzein, genistein, and their metabolites in gut contents and feces.** The concentrations of daidzein and genistein in the contents of all 3 gut segments (Fig. 1) and feces (Fig. 2) were lower in SIA rats than in control rats (*P* < 0.001). In both groups, the concentration of daidzein and genistein was considerably higher in the small intestinal contents than in cecal contents, colonic contents, and feces (*P* < 0.05). Although the diet contained similar amounts of both isoflavones, the concentrations of genistein in gut contents and feces exceeded those of daidzein (*P* = 0.005) except in fecal samples of SIA rats. Dihydrodaidzein and dihydrogenistein were detected in all gut segments and feces of the SIA rats (Figs. 1 and 2). However, the dihydrogenistein concentrations in gut contents were up to 426-fold higher than those of dihydrodaidzein (Fig. 1). Low amounts of dihydrodaidzein were also detected in small intestinal contents of male control rats (2.77 nmol/g wet weight), which did not differ from those of the male SIA rats (Fig. 1A). Low dihydrogenistein concentrations also occurred in small intestinal contents of both male (5.05 nmol/g wet weight) and female (1.35 nmol/g wet weight) control rats (Fig. 1D). But in this case, the dihydrogenistein concentrations were 24-fold higher in the SIA rats than in the control rats (*P* < 0.001). Equol and 5-hydroxy-equol were present in the contents of all three gut segments and the feces of the SIA rats but not in those of the control rats (Figs. 1 and 2). The equol concentrations largely exceeded those of 5-hydroxy-equol (*P* = 0.005) and male rats generally showed higher concentrations of both compounds (Figs. 1 and 2). Whereas the equol concentrations did not differ among the contents of gut segments and feces, the concentrations of 5-hydroxy-equol were higher in feces than in intestinal contents (*P* ≤ 0.005). This observation and the fact that the concentrations of dihydrogenistein and dihydrodaidzein also differed between the colonic contents and feces (*P* = 0.005) indicate further bacterial conversion during the 12-h collection period of fecal samples.

<table>
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<tr>
<th>Gut segment</th>
<th>SIA rats</th>
<th>Control rats</th>
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<tr>
<td></td>
<td><em>S. isoflavoniconvertens</em></td>
<td>Total bacteria</td>
</tr>
<tr>
<td>Small intestine</td>
<td>6.68 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.22 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Cecum</td>
<td>9.43 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3 ± 0.06&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon</td>
<td>9.44 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.0 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
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In *log<sub>10</sub>* cells/g dry weight.

<sup>a</sup> Values are means ± SD, *n* = 10. Means in a column with superscripts without a common letter differ, *P* < 0.01. *Different from counts of total bacteria in cecum of control rats, *P* = 0.006. SIA, *Slackia isoflavoniconvertens*-associated.

The percentage of free daidzein and genistein differed between SIA and control rats in a few samples only, with lower free isoflavone proportions being predominantly observed in the SIA rats (Supplemental Tables 2 and 3). Daidzein, genistein, and their metabolites occurred predominantly in their free form, apart from the small intestine (Supplemental Tables 2 and 3). Up to 3.4-fold higher proportions of free daidzein or genistein were detected in individual gut segments of female SIA and control rats compared to their male counterparts. Female SIA rats also had higher proportions of free dihydrogenistein than male SIA rats. Dihydrodaidzein and 5-hydroxy-equol occurred independently of gender exclusively in their free forms.

**Daidzein, genistein, and their metabolites in plasma and urine.** The plasma of the SIA rats contained daidzein, genistein, dihydrogenistein, and equol, whereas dihydrodaidzein and 5-hydroxy-equol were not detected (Fig. 3). The plasma concentrations of daidzein and genistein in the control rats were higher than those in the SIA rats and no daidzein or genistein metabolites were observed (Fig. 3). In the control group, the concentrations of daidzein and genistein were higher in male than in female rats (Fig. 3). In contrast, the daidzein concentration was higher in female than in male SIA rats (Fig. 3A). Daidzein, genistein, and their metabolites in the plasma occurred exclusively as glucurono- and/or sulfo-conjugates.

The control rats excreted large amounts of daidzein and genistein and low levels of dihydrodaidzein and dihydrogenistein in their urine (Fig. 4). The urinary daidzein and genistein concentrations of the SIA rats were considerably lower than those of the control rats (Fig. 4). Only the SIA rats excreted equol and 5-hydroxy-equol, with the levels of equol exceeding largely those of 5-hydroxy-equol (*P* = 0.005). Gender differences were observed within the SIA group; higher concentrations of daidzein, genistein, and dihydrodaidzein and lower concentrations of 5-hydroxy-equol occurred in female compared to male rats (Fig. 4). Urinary daidzein, genistein, and their metabolites appeared in their free and conjugated forms. Daidzein, genistein, and equol occurred predominantly as conjugates, whereas 5-hydroxy-equol was mainly present in its free form (Supplemental Tables 2 and 3). The genders differed in both the SIA and control groups. Except for 5-hydroxy-equol, the proportions of the free isoflavones and their products in the urine of male rats were 6- to 62-fold higher than those of female rats (Supplemental Tables 2 and 3).

**IGF-I in plasma.** The plasma IGF-I concentration did not differ between all SIA rats [1.20 (0.69–1.27) mg/L] and all control rats [0.99 (0.44–2.05) mg/L] or between female SIA rats [0.92 (0.69–1.24) mg/L] and female control rats [0.86 (0.44–1.27) mg/L] or male SIA rats [1.23 (1.18–1.27) mg/L] and male control rats [1.05 (0.89–2.05) mg/L]. However, the concentration in all female rats [0.99 (0.44–2.05) mg/L] was less than in all male rats [1.22 (0.89–2.05) mg/L] (*P* < 0.05). Differences between genders within the SIA and control groups were not significant because of the high variability (CV 3–41%) in medians (*P* = 0.056–0.222).

Discussion

To evaluate its in vivo activity, *S. isoflavoniconvertens* was introduced into SIHUMIx rats harboring a defined human intestinal microbiota. Preliminary in vitro tests showed that the...
SIHUMIx consortium is unable to convert daidzein or genistein. The SIHUMIx rat model mimics the basic microbiota composition of humans (18) and enabled the stable colonization of the rat’s intestine by *S. isoflavoniconvertens*. Previous attempts to monoassociate germfree rats with *S. isoflavoniconvertens* had been unsuccessful. The *S. isoflavoniconvertens* numbers in the large intestine of the SIA rats were 3 log₁₀ higher than the mean counts of *S. isoflavoniconvertens* strains reported for Japanese adults (6.4 log₁₀ cells/g fecal wet weight) (21). The bacterium was detected in 40% of the participants (21), which corresponds to the mean proportion of the equol-producing phenotype in the Japanese population of 48% (23).

The source of isoflavones in this study was the rats’ diet, which contained considerable amounts of daidzein and genistein similar to other soy protein-containing rodent diets (19). Compared to the application of pure compounds, this approach is more relevant for human nutrition, because the isoflavones are usually consumed within a food matrix. All rats associated with *S. isoflavoniconvertens* formed equol and 5-hydroxy-equol in the intestine. Moreover, the appearance of equol in plasma and the urinary excretion of equol and 5-hydroxy-equol indicated the absorption of these bacterial metabolites from the gut lumen. The mean plasma equol concentration of SIA rats was 5.2 μmol/L at an estimated daily intake of 42 mg daidzein/kg BW. Conventional rats were reported to have serum equol concentrations of up to 2.5 μmol/L in response to feeding 10 mg daidzein/kg BW with a soy protein-containing diet (24). Serum equol concentrations in humans ranged from 10 to 139 nmol/L after consumption of soy milk (25). The corresponding genistein metabolite, 5-hydroxy-equol, was not detected in the plasma of SIA rats and its urinary concentrations were considerably lower than those of equol. Nevertheless, in this study, we demonstrated for the first time, to our knowledge, the in vivo formation of 5-hydroxy-equol and its subsequent absorption.

In addition to the product formation, the pronounced in vivo metabolization of daidzein and genistein by *S. isoflavoniconvertens* was reflected by lower levels of these isoflavones in all samples of the SIA rats than in those of the control rats. Although the levels of both isoflavones in the diet were similar, the concentrations of daidzein in the intestinal contents of all rats were always lower than those of genistein. This difference may be explained by increased absorption and consequently higher bioavailability of daidzein (26–29).

**FIGURE 1** Total concentrations of daidzein, genistein, and their metabolites in contents of the small intestine (A, D), cecum (B, E), and colon (C, F) of male and female control and SIA rats. The middle line in the box plots shows the median, the bottom and top of the box are the 25th and 75th percentiles, the ends of the whiskers represent the 10th and 90th percentiles and the dots indicate outliers. n = 5. Differences between control and SIA rats: *p < 0.05, **P < 0.01; differences between male and female rats, *P < 0.05, **P < 0.01. DAI, daidzein; DHD, dihydrodaidzein; DHG, dihydrogenistein; EQ, equol; GEN, genistein; 5OHEQ, 5-hydroxy-equol; SIA, Slackia isoflavoniconvertens-associated.
Surprisingly, low levels of dihydrodaidzein and dihydrogenistein were detected in the small intestinal contents and urine of the control rats. Because neither of the two compounds was present in the diet, their formation most likely resulted from the activity of rat enzymes. The appropriate reduction may have been catalyzed by the quinone reductase type 1, a phase II detoxification enzyme known to be induced by isoflavones in rodents (30,31). Dihydrodaidzein and dihydrogenistein were also detected in the urine of even germfree rats fed a soy protein-containing diet (15) at similar levels to those observed for the control rats of our study.

The concentrations of daidzein, genistein, and their metabolites in intestinal contents and feces of female SIA rats were lower than in male SIA rats, indicating a more efficient isoflavone absorption in the females. The increased urinary excretion of daidzein, genistein, and dihydrodaidzein by female rats supports this conclusion. However, the urinary equol concentrations were similar in males and females.

Daidzein, genistein, and their metabolites occurred in cecal and colonic contents and feces predominantly or completely in their free form, whereas the small intestinal contents and urine were dominated by glucurono- and sulf-conjugates. During or after absorption, isoflavones are conjugated in intestine and liver by UDP-glucuronosyltransferases and to a lesser extent by sulfotransferases (32–34). The glucuronidation of isoflavones by small intestinal microsomes proceeds at a higher rate than that by colonic microsomes (35,36), which is in line with the greater proportion of conjugates in the small intestinal contents compared to cecal and colonic contents of the rats in the present study. The high bacterial counts in cecum and colon may have led to the hydrolysis of conjugates following biliary excretion into the gut lumen. Several members of the SIHUMIx consortium (B. longum, C. butyricum, and E. coli) harbor β-glucuronidase and/or sulfatase activity (37). The compounds detected in the plasma of the rats were completely conjugated, which is well known for flavonoids, including the isoflavones.
FIGURE 4 Total concentrations of daidzein and its metabolites (A) and genistein and its metabolites (B) in urine of male and female control and SIA rats. The middle line in the box plots shows the median, the bottom and top of the box are the 25th and 75th percentiles, the ends of the whiskers represent the 10th and 90th percentiles and the dots indicate outliers. *P < 0.05, **P < 0.01; differences between male and female rats: *P < 0.05, **P < 0.01. DAI, daidzein; DHD, dihydrodaidzein; DHG, dihydrogenistein; EQ, equol; GEN, genistein; SOHEQ, 5-hydroxy-equol; SIA, Slackia isoflavoniconvertens-associated.

(Daidsin and genistein in the urine of rats was also observed in a previous study (24). The proportion of conjugated daidzein, genistein, dihydrogenistein, and equol in the urine of female rats exceeded that determined for male rats, which may be explained by higher levels of certain UDP-glucuronosyltransferase isoforms in female rats (39,40).

Isoflavones have been associated with a decreased risk of hormone-dependent and age-related diseases, including cancer (1–3). One of the discussed mechanisms of cancer prevention is the reduction of the levels of IGF-I, which regulates cell proliferation, differentiation, and apoptosis. Increased circulating IGF-I concentrations are generally associated with increased breast and prostate cancer risk. However, the results of investigations on the relationship of isoflavone intake and plasma levels of IGF-I are still inconsistent (7). A negative association of the change in serum IGF-I concentrations with serum equol concentrations after isoflavone supplementation was observed in humans, suggesting that isoflavones might have an IGF-I-lowering effect exclusively in equol producers (41). In the present animal study, the plasma IGF-I concentrations were not reduced in the equol-forming SIA rats compared to the control rats. The lack of an effect may have been due to differences in regulation of the IGF system of rats and humans (42). Independent of the equol status, the values differed by gender, being lower in female rats. This is in agreement with data on gender differences in plasma IGF-I of rodents and humans at an advanced age (7,43,44). The analysis of biomarkers other than IGF-I in the SIA rats in future studies may allow to pinpoint the contribution of Slackia isoflavoniconvertens to the isoflavone effects.

In summary, Slackia isoflavoniconvertens formed equol in soy-fed gnotobiotic rats, indicating its contribution to the bioactivation of daidzein also in humans. The conversion of genistein resulted preferentially in the formation of dihydrogenistein, indicating that its further conversion to 5-hydroxy-equol was slowed down. This may explain why this compound has never been detected in vivo.

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**Literature Cited**