Isoflavonoid glucosides are deconjugated and absorbed in the small intestine of human subjects with ileostomies1–3

Kelly R Walsh, Sara J Haak, Torsten Bohn, Qingguo Tian, Steven J Schwartz, and Mark L Failla

ABSTRACT
Background: Although soy isoflavonoids have a number of health-promoting benefits, information concerning the sites of their absorption and metabolism in humans remains limited. Isoflavonoid absorption from the gut requires deconjugation of glucosides to aglycones.

Objective: The objective was to investigate the role of the small intestine in isoflavonoid absorption and metabolism in humans.

Design: Human subjects with fully functional gastrointestinal tracts (n = 6) and ileostomy subjects (n = 6) were fed a single soy meal containing 64.8 mg isoflavonoid aglycone equivalents (95% as glucosides). Metabolism of isoflavonoids in the upper gastrointestinal tract was examined by analyzing ileal effluent from ileostomy subjects, and absorption was assessed indirectly by quantifying isoflavonoids and several metabolites in 24-h urine pools.

Results: Chyme contained 36.7% of ingested isoflavonoid aglycone equivalents, primarily (95.8%) as aglycones. Qualitative profiles (x ± SEM) of isoflavonoid excretion in urine (daidzein > glycitein > genistein) and the quantity of isoflavonoid equivalents were not significantly different between the control (18.4 ± 2.2 mg) and ileostomy (13.5 ± 3.2 mg) subjects. Dihydroadaidzein was present in the urine of all subjects, although the amount excreted by ileostomy subjects was less than that excreted by the control subjects. The percentage of producers and mean quantities of dihydrodaidzein (DHD) and then converted to O-desmethylangolensin (O-Dma) or equol, whereas metabolites of genistein include dihydrogenistein (DHG) and 6'-hydroxy-O-desmethylangolensin (6-OH-O-Dma) (11, 12). These metabolites can be absorbed or further degraded in the lumen to phenolic compounds. More recently, glycitein has been shown to be converted to dihydroglycitein, which may be metabolized to 5'-methoxy-O-desmethylangolensin or dihydro-6,7,4'-trihydroxyisoflavone (13).

Conclusions: Ileostomy subjects efficiently deglycosylate isoflavonoid glucosides in the small intestine and appear to absorb aglycones with an efficiency comparable with that of control subjects. However, the production of microbial metabolites of isoflavonoids is limited in ileostomy subjects.

KEY WORDS Soy isoflavonoids, isoflavonoid absorption, ileostomy, small intestine, equol, dihydroadaidzein, dihydrogenistein, O-desmethylangolensin

INTRODUCTION
Isoflavonoids are phytoestrogens that exist predominantly as glucoside conjugates in soybeans and unfermented soy foods (1). Despite considerable interest in the health-promoting effects of isoflavonoids, information about their sites of absorption and metabolism in humans remains limited. It is well established that the absorption of dietary isoflavonoids requires deconjugation of isoflavonoid glucosides to aglycones (2). Plasma concentrations of phase II conjugates of isoflavonoid aglycones initially peak 1–2 h after the consumption of foods with soy and then again after 4–10 h (3–6). It is generally assumed that this increase in plasma isoflavonoid aglycones is due to the microbial hydrolysis of isoflavonoid glucosides in the large intestine (7, 8). This hypothesis is supported by the observation that the second peak in plasma isoflavonoids was absent after a human subject with markedly reduced gut microflora ingested a beverage containing isoflavonoid glucosides (9). Alternatively, the second rise in plasma isoflavonoids after the consumption of isoflavonoid glucosides may reflect enterohepatic recirculation (10). Isoflavonoid aglycones that are ingested or generated in the gastrointestinal tract also can be converted by the microflora to various metabolites. The aglycone daidzein can be reduced to dihydroadaidzein (DHD) and then converted to O-desmethylangolensin (O-Dma) or equol, whereas metabolites of genistein include dihydrogenistein (DHG) and 6'-hydroxy-O-desmethylangolensin (6-OH-O-Dma) (11, 12). These metabolites can be absorbed or further degraded in the lumen to phenolic compounds. More recently, glycitein has been shown to be converted to dihydroglycitein, which may be metabolized to 5'-methoxy-O-desmethylangolensin or dihydro-6,7,4'-trihydroxyisoflavone (13).

The active participation of the small intestine in the production and absorption of isoflavonoid aglycones has been supported by several reports. Andlauer et al (14, 15) showed that the isolated small intestine from rats deglycosylates isoflavonoid glucosides. Bowey et al (16) showed that daidzein and genistein were present in the urine of germ-free rats, which indicated that microbial activity is not essential for the deglycosylation of isoflavonoid glucosides and the absorption of the aglycones. Lactase phlorizin hydrolase in the brush border membrane of the small intestine is

1 From the Interdisciplinary PhD Program in Nutrition (KRW, SJS, and MLF), the Department of Human Nutrition (SJH and MLF), and the Department of Food Science (TB, QT, and SJS), The Ohio State University, Columbus, OH.
2 Supported by grant 2001-52102-11333 from the US Department of Agriculture, Cooperative State Research, Education, and Extension Service, the Ohio Agriculture Research and Development Center, and by the Initiative for Future Agriculture and Food Systems and the Virginia Vivian Graduate Student Research Scholarship (to SJH).
3 Reprints not available. Address correspondence to ML Failla, Department of Human Nutrition, The Ohio State University, 1787 Neil Avenue, 325 Campbell Hall, Columbus, OH 43210. E-mail: failla.3@osu.edu. Received August 20, 2006. Accepted for publication November 14, 2006.

capable of deglycosylating isoflavonoid glucosides (17–19). We previously showed that isoflavonoids from food are stable during simulated oral, gastric, and small intestinal phases of digestion, which suggests that conversion of isoflavonoid glucosides to aglycones is mediated by mucosal epithelial or microbial enzymes rather than by enzymes secreted by the exocrine pancreas (20).

In the present study, we investigated the gastrointestinal metabolism and absorption of isoflavonoids in human subjects with ileostomies. The gastrointestinal metabolism of isoflavonoids was assessed by HPLC analysis of ileal chyme. Isoflavonoid absorption in subjects with ileostomies was compared with that of control subjects with fully functional gastrointestinal tracts by quantifying deconjugated isoflavonoid aglycones and metabolites in urine (21).

SUBJECTS AND METHODS

Supplies

Standards for daidzein, genistein, glycine, and their β-, acetyl- and malonyl-glucosides were purchased from LC Laboratories (Woburn, MA). 2′,4′-Dihydroxy-2-phenylacetophenone (internal standard) was purchased from the Indofine Chemical Company, Inc (Hillsborough, NJ). β-D-Glucuronide glucuronosylhydrolyase (β-glucuronidase) type H-5 from Helix pomatia (G-1512) and 2-phenyl-4H-1-benzoazepine-4-one (flavone, internal standard) were purchased from Sigma Chemical Co (St. Louis, MO). Nylon syringe filters (0.2 and 0.45 μm) and C18 extract-clean solid-phase extraction columns (no. 205462) were purchased from Alltech Associates Inc (Deerfield, IL). Powdered isoflavonoid extract was generously donated (Advantogy Clear; Cargill, Wayzata, MN). Condensed tomato soup (Campbell Soup Company, Camden, NJ), bovine milk (2% fat), and soymilk (Westsoy Plus, Garden City, NJ) were purchased from a local grocery store. All other supplies were purchased from Fisher Scientific Co (Fairlawn, NJ). All reagents used for HPLC and mass spectrometry (MS) analysis were HPLC grade.

Subjects

Human subjects included individuals with ileostomies (n = 6) and control subjects with intact gastrointestinal tracts (n = 6). Ileostomy subjects (4 men and 2 women aged 63.7 ± 5.8 y; range: 47–84 y) underwent proctocolectomy for ulcerative colitis 13.5 ± 5.2 y (range: 3–36 y) before this study. Two of the subjects with ileostomies had internally implanted J-pouches requiring intubation to eliminate ileal effluent, whereas the other ileostomy subjects had external pouches. Control subjects were age- and sex-matched to the ileostomy subjects (4 men and 2 women aged 64.0 ± 7.8 y; range: 49–84 y). Inclusion criteria required that all subjects were otherwise healthy, had no history of nutrient malabsorption, were ≥18 y of age, were lactose tolerant (self-reported), were not regular consumers of soy, and had not used antibiotics for ≥6 mo before the study. The subjects completed an initial screening that included a food-frequency questionnaire addressing their soy-consumption patterns (22). Written informed consent was obtained before participation in the study.

Study design

The study was approved by The Ohio State University Institutional Review Board and was conducted over 2 d. On day 1, the subjects were instructed to collect all of their urine (baseline), avoid eating soy-containing foods for the duration of the study, and record all foods and beverages consumed. The subjects were counseled by a trained nutritionist and provided with a list of common soy foods as a guide for foods to avoid. Specimen vessels containing L-ascorbic acid (1.14 mmol) and boric acid (4.85 mmol) as preservatives were provided for pooling urine (23).

On day 2, the subjects consumed a soy test meal at 0700 that consisted of one serving (236 mL) of tomato soup prepared with condensed tomato soup (118 mL), bovine milk (59 mL) with 2% fat, and soymilk (59 mL) and fortified with 125 mg isoflavonoid extract. The subjects also ingested 2 dinner rolls without soy ingredients to clean the contents of the soup bowl, orange juice (177 mL), and coffee (if desired) with the test meal. Individuals were instructed to refrain from eating for a minimum of 4 h after consuming the test meal. The subjects (n = 12) collected all urine for 24 h after the test meal including the first morning void the following day, and ileostomy subjects (n = 6) also collected ileal effluent for 24 h after the meal and recorded the collection times. All samples were stored on ice until retrieved later that morning by the investigators and were then stored at −20 °C until analyzed. Ileal effluent samples were weighed and homogenized with a blender before analysis for isoflavonoids.

Extraction of isoflavonoids from meal and ileal effluent samples

Isoflavonoids were extracted from the soy test meal and ileal effluent samples as previously described (20, 24). These samples were not treated with β-glucuronidase or sulfatase from Helix pomatia because β-glucosidase activity has been reported for this enzyme preparation (2). Aliquots (0.25 g) of sample were mixed with 0.1 mol HCl/L (1 mL), acetonitrile (5 mL), and water (1.5 mL). Samples were spiked with a stock solution of flavone (50 μL) in 96% ethanol in water (by vol) as an internal standard (25). The mixture was shaken (Thermolyne Vari-Mix Shaker; Barnstead/Thermolyne, Dubuque, IA) slowly at room temperature for 2 h. Samples were centrifuged (Fisher Centrifuge; Fisher Scientific) at 450 × g for 30 min at room temperature, and aliquots (1 mL) of the supernatant fluids were dried under nitrogen at room temperature. Dried residues were resublimed in 80% methanol: 20% water and filtered (0.2 μm) before HPLC injection.

Extraction of isoflavonoids and isoflavonoid metabolites from urine samples

Isoflavonoids were extracted from urine samples by using a modification of the method of Kulling et al (26). Aliquots of pooled 24-h urine samples (8 mL) from each subject were centrifuged (Avanti J-25, Beckman Coulter Inc, Palo Alto, CA) at 10 000 × g for 10 min at 4 °C. Aliquots (5 mL) of the supernatant fluids were filtered (0.45 μm) into polypropylene tubes, and sodium acetate buffer (1.5 mL, pH 4.0) was added. Samples were spiked with 2′,4′-dihydroxy-2-phenylacetophenone (final concentration: 25 μmol/L) in chloroform as an internal standard. C18 solid-phase extraction columns attached to a vacuum manifold (no. 210351; Alltech Associates) were conditioned with methanol (6 mL) followed by sodium acetate buffer (6 mL, pH 4.0). Solid-phase extraction columns were continually monitored to
prevent them from running dry during the procedure. Urine samples were drawn through the columns, which were then sequentially washed with sodium acetate buffer (4 mL, pH 4.0), water (1 mL), and methanol (1 mL). Isoflavonoids and their derivatives were then eluted into glass vials with methanol (7 mL). Samples were dried under nitrogen at 37 °C and resolubilized in buffer (1 mL) containing β-glucuronidase (200 units/mL in sodium acetate buffer, pH 5.0). Samples were incubated in a shaking (50 rpm) water bath (Versa-Bath S Model 224; Fisher Scientific) for 18–22 h at 37 °C. After the addition of diethyl ether (3.5 mL) and vigorous mixing, the organic phases were transferred to glass vials, and the extraction with diethyl ether was repeated. Extracts were dried under nitrogen at room temperature, resolubilized in 80% methanol:20% water (0.6 mL), and filtered (0.2 μm) before injection.

**HPLC analysis**

Isoflavonoid profiles of samples were determined by using a Waters 2695 HPLC (Milford, MA) combined with a Waters 2996 photodiode array detector. Separation was achieved by using a Waters Nova-Pak C18 reversed-phase column (150 mm × 3.9 mm internal diameter, 4 μm, 60 Å pore size) with an inline Nova-Pak C18 guard column (20 mm × 3.9 mm internal diameter, 4 μm) for the stationary phase. The sample injection volume was 10 μL, and data were collected over the spectral range of 210 to 400 nm. For isoflavonoids extracted from meal and ileal effluent samples, a mobile phase of 1.0% acetic acid in water (by vol; solvent A) and acetonitrile (solvent B) with the following linear solvent gradient at 25 °C ± 5 °C was used: 0 to 5 min, 15% B; 5 to 36 min, 15% to 29% B; 36 to 44 min, 29% to 35% B; 44 to 45 min, 35% to 85% B; and, 45 to 50 min, 85% to 15% B. A flow rate of 0.6 mL/min was maintained throughout the run. For isoflavonoids extracted from urine samples, the mobile phase consisted of 1.0% acetic acid in water (by vol; solvent A), acetonitrile (solvent B), and methanol (solvent C) at a constant flow rate of 0.55 mL/min. The following linear solvent gradient was used at 25 °C ± 5 °C to elute isoflavonoids and their derivatives: 0 to 1 min, 75% A and 12% B; 1 to 14 min, 75% to 49% A and 12% to 25% B; 14 to 15 min, 49% to 10% A and 25% to 45% B; 15 to 19 min, no change; 19 to 20 min, 10% to 75% A and 45% to 12% B; and, 20 to 25 min, no change.

**Quantification of isoflavonoids**

Isoflavonoids were identified on the basis of retention times and ultraviolet absorption spectra of pure (≥98%) isoflavonoid standards. Stock solutions of pure isoflavonoid standards in 80% methanol: 20% water were serially diluted to prepare standard curves with concentrations calculated by using the Beer-Lambert law and published absorbance maxima and molar extinction coefficients (27). Each working solution was analyzed by HPLC to generate external calibration curves that correlated HPLC peak areas with calculated concentrations. Samples were analyzed in duplicate and data are expressed as means. HPLC could not be used to quantify daidzein in urine from one of the ileostomy subjects because of the presence of a coeluting peak. Therefore, the daidzein content of that urine sample was determined by HPLC-MS/MS as described below.

**HPLC-MS/MS analysis**

Analysis for the major isoflavonoid aglycone metabolites in urine samples was performed by HPLC-MS/MS. HPLC conditions were identical to those used for urine analysis. MS was performed by using positive-ion electrospray ionization on a triple quadrupole mass spectrometer (Micromass Co, Ltd, Manchester, United Kingdom). Selected reaction monitoring was used for detection of the isoflavonoid metabolites DHQ [mass-to-charge ratio (m/z) 239 > 123], DHG (m/z 273 > 123), 0-Dma (m/z 259 > 121), equol (m/z 243 > 133), and daidzein (m/z 255 > 199). These transitions were determined while scanning for the most abundant daughter ions of the respective standards. Working conditions of the mass spectrometer were as follows: capillary voltage, 3.0 kV; cone voltage, 35 V; radio-frequency lens 1, 50 V; source temperature, 120 °C; desolvation gas temperature, 500 °C at a flow of 16.3 L/min; and collision energy, 25 eV.

**Statistical analysis**

All statistical analyses were performed by using SPSS release 14.0 for WINDOWS (SPSS Inc, Chicago, IL). Data are expressed as means ± SEMs when appropriate. Isoflavonoid excretions between the control group and the ileostomy subjects were compared by using a general linear multivariate model, with group (control, ileostomy) as a fixed factor and the amount of excreted isoflavonoids (total, daidzein, glycitein, genistein) as the dependent variables. Normal distribution of isoflavonoid excretion in urine and effluent was examined by using normality plots and Kolmogorov-Smirnov tests. Equality of variance between control and ileostomy subjects was verified by Levene’s test and box plots. Analyses for correlation were performed by linear regression, and Pearson correlation coefficients were calculated. Differences were considered statistically significant when the P value was <0.05.

**RESULTS**

Unless otherwise stated, all data are presented as isoflavonoid aglycone equivalents. All subjects indicated (by self-report) that they were not regular consumers of soy products and avoided consuming soy-containing products for the duration of the study. Review of diet records by a registered dietitian confirmed that subjects were compliant in avoiding soy foods before and after administration of the test meal. The soy test meal administered to human subjects contained 64.8 mg isoflavonoid aglycone equivalents (Table 1) with ≈40 mg contributed by the isoflavonoid extract and the remaining 25 mg by the soymilk. Daidzin, glycitin, genistin, and malonylgenistin accounted for ≈94.7% of total isoflavonoids. Daidzin was the most abundant isoflavonoid in the meal, representing 45.7 ± 0.7% of total isoflavonoids. The glycitin content of the test meal was slightly higher than that of genistin (16.6 and 14.6 mg isoflavonoids, respectively) and more abundant (25.6 ± 0.8% of total isoflavonoids) than that typically present in most soy foods (1). This was due to the abundance of glycitin in the isoflavonoid extract. Malonylgenistin was the only acylated glucoside detected in the test meal. Isoflavonoid aglycones accounted for ≈5.2% of total isoflavonoids in the meal. Total isoflavonoids in the ileostomal effluent (Figure 1) varied widely, ranging from 1.3 to 46.3 mg (23.8 ± 6.4 mg; 36.7 ± 9.9% of the test meal). Isoflavonoid glucosides accounted for
ISOFLAVONOID METABOLISM AND ABSORPTION IN HUMANS

TABLE 1
Isoflavonoid glucoside (IFG) and aglycone (IFA) contents of the soy test meal

<table>
<thead>
<tr>
<th>Isoflavonoid</th>
<th>Glucoside</th>
<th>Aglycone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/meal</td>
<td>mg/meal</td>
</tr>
<tr>
<td>Daidzein</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Daidzin</td>
<td>48.5 ± 1.2</td>
<td>29.6 ± 0.7</td>
</tr>
<tr>
<td>Acetyldaidzin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Malonyldaidzin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glycitein</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Glycitin</td>
<td>26.0 ± 0.8</td>
<td>16.6 ± 0.5</td>
</tr>
<tr>
<td>Acetylglycitin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Malonylglycitin</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Genistin</td>
<td>23.4 ± 1.0</td>
<td>14.6 ± 0.6</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Daidzin</td>
<td>23.4 ± 1.0</td>
<td>14.6 ± 0.6</td>
</tr>
<tr>
<td>Glycitein</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Malonyldaidzin</td>
<td>1.4 ± 0.6</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Total</td>
<td>102.7</td>
<td>64.8</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; n = 7. ND, not detected.
2 Aglycone equivalent = (IFA molecular weight/IPG molecular weight) × (IFG mass).

94.8 ± 2.5% of the total isoflavonoids in the test meal, but only 4.2 ± 2.1% in the ileal effluent. Free aglycones were quantitated in ileal effluent because samples were analyzed without incubation with β-glucuronidase and sulfatase. The quantities of genistein (8.85 ± 3.1 mg) and daidzein (8.5 ± 2.4 mg) in effluent were greater than glycitein (5.5 ± 1.4 mg). However, effluent contained a greater (P = 0.05) percentage of ingested genistein (55.0%) than ingested daidzein (27.7%) or glycitein (30.6%). Genistein (1.0 ± 0.8 mg genistein equivalents) was present in the effluent of 2 subjects, but daidzin and glycitin were not detected in effluent from any subject. The isoflavonoid metabolites DHD, DHG, equol, O-Dma, and 6-OH-O-Dma were not detected in any of the samples of ileal effluent.

Isoflavonoids were not detected in the baseline urine samples of control or ileostomy subjects, which confirmed their self-reported avoidance of soy products. After ingestion of the soy-containing meal, total isoflavonoids in urine (Figure 2) from control subjects (18.4 ± 2.2 mg; 28% of ingested dose) exceeded that for subjects with ileostomies (13.5 ± 3.2 mg; 20.9% of ingested dose); however, the difference was not significant (P = 0.233). The profile of isoflavonoids excreted during the 24-h period after ingestion of the meal was consistent for control and ileostomy subjects; the greatest amounts were for daidzein (15.9 ± 2.1 and 9.4 ± 2.5 mg/24 h, respectively) followed by glycitein (1.6 ± 0.4 and 2.6 ± 1.0 mg/24 h, respectively) and genistein (0.9 ± 0.3 and 1.5 ± 0.7 mg/24 h, respectively) in enzyme-treated urine. This pattern of isoflavonoid excretion (ie, daidzein > glycitein > genistein) was observed in 10 of 12 subjects. A trend for higher daidzein excretion in control subjects than in ileostomy subjects was observed (P = 0.076).

Excretion of isoflavonoids in urine during the 24-h period after ingestion of the meal was ingested ranged from 12.9 to 27.0 mg for control subjects and from 3.2 to 22.4 mg for ileostomy subjects (Figure 3). Genistein was not detected in enzyme-treated urine from one ileostomy subject and from one control subject, and glycitein was not detected in urine from one control subject. The quantities of glycitein (r² = 0.56, P = 0.09) and genistein (r² = 0.50, P = 0.12) in enzyme-treated urine were inversely correlated with the amount of their respective aglycone equivalents in the ileal chyme, but were not statistically significant. The amount of daidzein in ileal chyme showed no correlation with the amount of daidzein present in the urine.

Several of the microbial metabolites of isoflavonoids were detected in the urine from the control subjects (Table 2). DHD and DHG were present in urine from all control subjects, whereas O-Dma and equol were present in the urine from 4 and 3 of these subjects, respectively. O-Dma and equol were detected in the urine from only one ileostomy subject. Urine from all ileostomy subjects contained minimal amounts of DHD compared with that from the control subjects. Three of the 6 ileostomy subjects excreted detectable, albeit low, amounts of DHG (2.6 ± 1.1 μg) in the 24-h urine sample. 6-OH-O-Dma was not detected in urine from any subject.

DISCUSSION
We examined the role of the small intestine in the digestion and absorption of isoflavonoid glucosides in human subjects with ileostomies. Ileostomy subjects have previously been used to...
examine the gastrointestinal metabolism and absorption of bioactive compounds, such as quercetin and β-carotene, in the upper gastrointestinal tract (28–31). Our results show for the first time that humans lacking a large intestine absorb isoflavonoids from soy food with an apparent efficiency that does not differ from that of individuals with an intact gastrointestinal tract. Analysis of ileal chyme showed that dietary isoflavonoid glucosides are efficiently hydrolyzed to aglycones before reaching the terminal ileum in ileostomy subjects. Our results also suggest that microorganisms inhabiting the small intestine of ileostomy subjects produce minimal, if any, DHD, DHG, O-Dma, and equol from isoflavonoid aglycones.

More than one-third of the ingested isoflavonoids were present in the ileal effluent; aglycones accounted for 95% of the total. The experimental design precludes evaluation of the relative contributions of lactase phlorizin hydrolase in the mucosal epithelium and microbial β-glucosidases in hydrolyzing the ingested isoflavonoid glucosides in the small intestine. It is unclear whether the relative abundance of the aglycones reflects a lack of direct coupling of hydrolysis of isoflavonoid glucosides with aglycone uptake into the intestinal epithelia or cleavage of phase II metabolites that reenter the luminal cavity via enteric and enterohepatic circulation (10, 32).

Isoflavonoids in urine are a marker of the ingestion of soy foods and a noninvasive indicator of bioavailability (33, 34). The qualitative profile of urinary isoflavonoids in enzyme-treated samples did not differ between control and ileostomy subjects. Daidzein was the predominant isoflavonoid in urine from 11 of 12 subjects. The ratio of daidzein equivalents to genistein plus genistein equivalents in the meal (≈1:1) and in urine (≈3:1 and 6:1 for ileostomy and control subjects, respectively) indicated that the relative extent of urinary excretion of daidzein exceeded that of genistein and genistein as observed previously in subjects with intact gastrointestinal tracts (11, 12). Also, the quantities of isoflavonoids in the urine of individuals with ileostomies varied widely, as did that of control subjects in this study and previous studies (8, 12). Differences in gut transit time, the activity of lactase phlorizin hydrolase, qualitative and quantitative profiles of intestinal microflora, absorptive efficiency, and postabsorptive metabolism of isoflavonoids likely contributed to the variation in the amounts of isoflavonoids excreted by individuals fed the identical meal (12, 35). There were marked differences in the urinary profile of microbial metabolites of isoflavonoids between the control subjects and the ileostomy subjects. Compared with the control subjects, much lower amounts of DHD and DHG were present in the urine of ileostomy subjects. Only one of the subjects with an ileostomy had microflora that appeared to be capable of producing O-Dma and equol. Urinary output of O-Dma by this individual exceeded that of the 4 control subjects excreting this metabolite. This individual had an internal J-pouch for 20 y rather than a stoma for external collection bags. In contrast, the metabolite profile in the urine of the second subject with the J-pouch (10 y since surgery) was similar to that of the 4 ileostomy subjects with external pouches.

In the present study, ≈37% and 21% of the isoflavonoids from the test meal were recovered in the ileal effluent and urine, respectively, of subjects with ileostomies. Fecal samples were not collected from control subjects because only a minor fraction of the ingested dose of isoflavonoids is eliminated by this route (12). Thus, we were unable to account for ≈40% of isoflavonoids ingested by subjects with ileostomies during the 24-h collection period. The amount of total isoflavonoids in the ileal effluent may be underestimated because phase II metabolites of the absorbed compounds present in bile and effluxed from the intestinal epithelium into the lumen would not be detected by our method of analysis (10, 32). In subjects with ileostomies, transit time through stomach and small intestine and ileal excretion of fat, protein, bile acids, and vitamin B-12 have been reported to be

### TABLE 2

Isoflavonoid metabolites in urine from control and ileostomy subjects

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control subjects</th>
<th>Ileostomy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of producers</td>
<td>6 of 6</td>
<td>6 of 6</td>
</tr>
<tr>
<td>Concentration (μg/24 h)</td>
<td>442.8 ± 126.7²</td>
<td>2.6 ± 1.3</td>
</tr>
<tr>
<td>Range</td>
<td>188.6–914.3</td>
<td>0.3–8.3</td>
</tr>
<tr>
<td><strong>DHG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of producers</td>
<td>6 of 6</td>
<td>3 of 6</td>
</tr>
<tr>
<td>Concentration (μg/24 h)</td>
<td>90.1 ± 24.8</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>Range</td>
<td>11.5–142.6</td>
<td>1.3–6.3³</td>
</tr>
<tr>
<td><strong>O-Dma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of producers</td>
<td>4 of 6</td>
<td>1 of 6</td>
</tr>
<tr>
<td>Concentration (μg/24 h)</td>
<td>22.0 ± 9.5</td>
<td>224.3</td>
</tr>
<tr>
<td>Range</td>
<td>30.0–47.8</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Equol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of producers</td>
<td>3 of 6</td>
<td>1 of 6</td>
</tr>
<tr>
<td>Concentration (μg/24 h)</td>
<td>475.8 ± 447.7</td>
<td>135.4</td>
</tr>
<tr>
<td>Range</td>
<td>223.2–2265.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

¹ NA, not applicable; O-Dma, O-desmethylangolensin.
² x ± SEM (all such values).
³ Range in 24-h urine samples from producers.
similar to that excreted in feces by normal subjects (36). However, the ileal mucosa is hypertrophied (37), and there is as much as an 80-fold increase in the ileal bacterial population after proctocolectomy (38, 39). These factors may affect the extent of metabolism of ingested isoflavonoids. The absence or low quantities of isoflavonoid metabolites in urine suggests that ileostomy subjects generally lack sufficient types or numbers of bacteria capable of producing detectable quantities of some of the metabolites. This possibility is supported by the observation that antibiotic-mediated reductions in gut microflora were associated with decreases in plasma equol and microbial metabolites in the urine of primates and a human subject, respectively (9, 40). Minimal production of DHD, DHG, O-Dma, and equol by the ileostomy subjects suggests that they may not benefit from the proposed health-promoting activities of these isoflavonoid metabolites (41, 42). Future studies in subjects with ileostomies should consider this possibility as well as the contributions of the intestinal metabolism of ingested isoflavonoids to phenolic acids and other compounds not investigated in the present study, hydrolysis of glucosides within stomal bags and pouches, and postabsorptive retention and metabolism in tissues.

Sincere thanks are extended to the participants in this study, John Smreczanski for his assistance with identifying the ileostomy subjects, Charles Cook for consultation on appropriate exclusion criteria for the subjects, and Carolyn Gunther for her critical review and editing of the manuscript.

KRW and SJH contributed equally to this project. SJH and MLF designed the study. SJH was responsible for recruiting and instructing the subjects, administering the test meals, and collecting the samples. KRW and SJH developed the test meal and were responsible for the HPLC analyses. KRW and SJH are registered dietitians and were responsible for the critical analysis of the diet records. QT, TB, and SJS analyzed and quantitated the isoflavonoid metabolites by HPLC-MS/MS. SJH, KRW, and MLF were responsible for data compilation and interpretation. TB provided the statistical analysis of the data. KRW prepared the initial draft of the manuscript. KRW and TB prepared revisions after critical input by the coauthors. MLF was the primary investigator and oversaw the entire study and the preparation of the report. None of the authors had financial or contractual conflicts of interest associated with this study.

REFERENCES