Rapid Gut Transit Time and Slow Fecal Isoflavone Disappearance Phenotype Are Associated with Greater Genistein Bioavailability in Women

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ABSTRACT The bioavailability of soybean isoflavones varies widely among individuals due to many factors, including activities of gut microflora. To characterize factors that affect fecal isoflavone disappearance phenotype and isoflavone bioavailability in women, 35 Asian and 33 Caucasian women, 18–43 y of age, provided fecal samples for anaerobic incubation with isoflavones in vitro at two times 5 mo apart (Phases I and II). Diet, physical activity and health history were investigated at these times. A single dose of soymilk powder [1.2 mg (4.57 μmol) total isoflavone/kg body] was given to all subjects with breakfast in phase II. Daidzein and genistein from fecal incubations, urine and fecal samples were measured by reverse-phase HPLC. Three significantly different daidzein and two genistein disappearance phenotypes were identified from fecal isoflavone incubations. More Asians than Caucasians were identified within the high daidzein disappearance phenotype. Caucasians and Asians differed significantly in daily intake of red meat (0.3 ± 0.1 vs. 1.0 ± 0.1 servings/d), dairy foods (2.9 ± 0.2 vs. 1.2 ± 0.2 servings/d) and insoluble dietary fiber (3.6 ± 0.3 vs. 1.4 ± 0.3 g). BMI, maximal oxygen uptake (VO_{2\text{max}}) and physical activity level were significantly greater in Caucasians than in Asians. Asian subjects of the low genistein disappearance phenotype had more rapid gut transit time (GTT) and greater isoflavone bioavailability as reflected in urinary genistein excretion than did Asians of the high genistein disappearance phenotype (GTT, 40 ± 8 vs. 63 ± 5 h; 11.0 ± 2.7 vs. 4.0 ± 1.7% of ingested genistein excreted in urine). Caucasians of both genistein disappearance phenotypes had longer GTT than did Asian subjects (84 ± 5 vs. 56 ± 6 h) and resembled Asians of the high genistein disappearance phenotype in genistein bioavailability. Relatively rapid GTT coupled with a low fecal isoflavone disappearance phenotype as occurred in Asian but not Caucasian subjects produced greater genistein bioavailability, as reflected in urinary genistein excretion. J. Nutr. 133: 3110–3116, 2003.

KEY WORDS: isoflavone • genistein • degradation • gut transit time • ethnicity

Isoflavones (daidzein, genistein, and glycitein) are diphenoic phytoestrogens in soy foods that may have beneficial health effects in humans. Crouse et al. (1) demonstrated that soy protein isolates containing ≥35 mg isoflavones/d reduced human plasma concentrations of total and LDL cholesterol in mildly hypercholesterolemic subjects. Alekel et al. (2) showed that soy protein containing isolavones contributed to bone health in perimenopausal women by reducing lumbar spine bone loss. Previous studies in our laboratory showed that the bioavailability of isoflavones varied widely among individuals, which is likely to influence the health effects of these compounds. When various doses of soymilk isoflavones were fed to women in a randomized crossover design, fecal isoflavone excretion of two subjects was 10–20 times greater than that of the other five subjects. Urinary isoflavone excretion of the two subjects who excreted large amounts of fecal isoflavones was two to three times greater than that of subjects excreting lesser amounts (3). Several factors may contribute to this variation. Hendrich et al. (4) showed three distinct isoflavone disappearance phenotypes (high, n = 5; moderate, n = 10; and low, n = 5) based on isoflavone disappearance half-life in anaerobically incubated fecal samples from 9 men and 11 women. The three isoflavone disappearance phenotypes were stable in 12 of 15 subjects reexamined after 10 mo. These results suggested that gut microorganisms play an important role in the metabolism of isoflavones. Clostridium, Butyrivibrio and Bacteroides species cleave the C-ring of flavonoids and isoflavonoids (5–7). Human gut microorganisms may vary significantly and stably to influence isoflavone degradation.
Diet, physical activity, and gut transit time (GTT)\(^4\) may influence the metabolic activity of gut microorganisms. Stephen et al. (8) showed that dietary fiber intake negatively correlated with GTT and bacterial mass (\(r = -0.77, P < 0.001\)). Physical activity may affect bowel habits, thus affecting activity of gut microorganisms. Bowel movements are more frequent in physically active people (9) and diarrhea may occur in long distance runners (10). But these factors are not well characterized. In the present study, we identified in vitro fecal isoflavone disappearance phenotypes, characterized phenotypic stability over 5 mo and compared isoflavone disappearance in Asian and Caucasian women. We also investigated whether diet, exercise and GTT were related to isoflavone disappearance phenotype and which of these factors influenced the apparent bioavailability of isoflavones as reflected in urinary isoflavone excretion.

SUBJECTS AND METHODS

**Subjects.** Thirty-five Asian (35 Chinese: 33 from China, 1 from the United States, 1 from Indonesia) and 33 Caucasian women (all from the United States) between 18 and 43 y of age were recruited from Iowa State University and the surrounding community. On the basis of health and medical history questionnaires in phases I and II, subjects were excluded if they had gastrointestinal, cardiovascular, liver, kidney or thyroid disease, food allergies, ethanol intake greater than 1 drink/d (360 mL beer, 120 mL wine or 45 mL hard liquor), smoked, used antibiotics within the past 3 mo, or regularly used prescription or nonprescription medication. The subjects were omnivorous except for one Asian pure vegetarian. The experimental procedures for this study were approved by the Human Subjects Committee of Iowa State University. Subjects gave their informed consent to the protocol.

**Experimental design.** The experiment was divided into phases I and II, 5 mo apart, to determine the phenotypic stability of isoflavone disappearance. In each phase, subjects provided freshly voided fecal samples for in vitro incubation with isoflavones. Two Caucasian subjects dropped out of the study due to graduation and a health problem before phase II. The following assessments were conducted for each subject in both phases: health and medical history questionnaires, Seven-Day Physical Activity Recall (11), body fat content by total body electrical conductivity, fitness by maximal oxygen uptake (\(VO_2 max\)) and dietary intake evaluation by food-frequency questionnaire (Nutritionist V, version 1.5, 1998; First DataBank, San Bruno, CA). A single dose of soy isoflavone was given in phase II. Soymilk powder (Now Foods, Glendale, IL) provided 1.2 mg total isoflavone/kg body (4.6 \(\mu\)mol isoflavone aglycone equivalents/kg: daidzein 2.3 \(\mu\)mol; genistein 2.2 \(\mu\)mol; glycitein 0.1 \(\mu\)mol). Subjects were instructed to avoid isoflavone-containing foods for 3 d before this treatment; a list of such foods was provided. After an overnight fast, 66 subjects consumed soymilk powder with breakfast between 0730 and 0830 h in the Human Metabolic Unit at Iowa State University. Two gelatin capsules containing 16 glass marker beads were given at the same time just before soymilk powder feeding. Urine was collected continuously for 24 h after soymilk feeding, then pooled and mixed. After recording the total volume, a 50-mL aliquot of each sample was stored at -80°C until analysis. After ingestion of 16 encapsulated glass beads with breakfast, feces were collected until 12 or more beads had been excreted for measuring GTT and fecal isoflavones (12). Feces were freeze-dried and after recording the total weights of dry samples, feces were ground to a fine powder in a coffee mill (Braun Company, Lynnfield, MA). Fecal samples (50 g) were stored at -80°C until analysis.

**In vitro anaerobic fecal sample incubations.** Brain heart infusion (BHI; 100 mL) medium (DIFCO Laboratories, Detroit, MI) consisted of 3.7 g BHI, 93 mL distilled deionized water (DD H2O), 5 mL 80 g/L NaCl, 2 mL of 12.5 g/L cysteine sulfide (reducing agent) and 0.1 mL of 1 g/L resazurin (O2 indicator, both from Aldrich Chemical, Milwaukee, WI). The medium was autoclaved at 121°C for 45 min. Daidzein and genistein were dissolved in BHI culture medium at concentrations of 600 \(\mu\)mol/mL. This standard solution was autoclaved under the same condition as the culture medium and sonicated for 10 min before use. A freshly voided fecal sample (3 g) from each subject was diluted 10-fold with 27 mL sterilized BHI culture medium in a culture tube and homogenized for 10 min using a Deluxe Mixer (American Scientific Products, McGraw Park, IL) under anaerobic conditions; 5 mL of this mixture was removed as negative control (contained only feces and medium) and 5 mL of isoflavone standard solution was added to the culture medium. The final concentration of each isoflavone in the culture tube was 100 \(\mu\)mol/mL. The mixture was vortexed for 5 min and the culture tube was incubated at 37°C for 24 h. At 0, 6, 12 and 24 h, 4 mL of culture mixture was removed anaerobically and stored at -80°C until analysis. Tubes containing 25 mL of sterile BHI medium and 5 mL isoflavone standards were incubated as positive controls. Positive and blank controls were incubated and sampled at the same time intervals as were fecal samples.

**Analytical methods**

**Fecal incubation sample preparation.** Sep-Pak C18 cartridges (Waters, Rainin, Woburn, MA) were activated by 5 mL methanol, then 5 mL DD H2O. Culture medium (2 mL) was slowly loaded on the preactivated Sep-Pak C18 cartridge, which was washed twice with 2 mL DD H2O. Isoflavones were eluted in 2 mL methanol/H2O (80:20 v/v), then filtered through a 0.45-\(\mu\)m polytetrafluoroethylene filter (Alltech Associates, Deerfield, IL) and analyzed in duplicate for isoflavone content by HPLC (see below).

**Fecal and urine analyses.** Urine sample preparation was modified from Zhang et al. (14). Urine, \(\beta\)-glucuronidase/sulfatase (H2 type, Sigma, St. Louis, MO), and 50 \(\mu\)L THB (2 g/L, internal standard) were incubated at 37°C for 18 h. Freeze-dried, ground feces (2 g) were extracted in duplicate with 10 mL acetonitrile, 2 mL of 0.1 mol/L HCl for 2 h at room temperature on a shaker; then extracts were filtered through No.1 filter paper (Whatman International, Maidstone, UK), and dried on a rotary evaporator at <30°C. HPLC analyses for isoflavones in urine, feces, and fecal incubations were as described (14).

**Soybean milk powder analysis.** The concentrations of total isoflavones in soymilk were measured by HPLC as previously described (15,16). The total isoflavone content was the sum of molar amounts of total daidzein, genistein and glycitein normalized to the aglycone forms.

**Recovery studies.** Positive controls from various time points were used to measure recovery of in vitro fecal incubation samples in duplicate. The recoveries of in vitro fecal daidzein and genistein

\(^4\) Abbreviations used: BHI, brain heart infusion; DD H2O, distilled deionized water; \(D_o\), daidzein disappearance rate constant; \(G_o\), genistein disappearance rate constant; GTT, gut transit time; THB, 2,4,4'-trihydroxybenzoic; \(VO_2 max\), maximal oxygen uptake.
incubation were 74.2 ± 5.0 and 77.6 ± 3.0%, respectively. Blank urine and fecal samples from one subject were collected for urinary and fecal recovery measurement. THB (50 μL) and different amounts of external daidzein and genistein standards (6.25–100 μmol/L) in duplicate were added during urine and fecal extractions. Recoveries of urinary daidzein, genistein and THB were 82.2 ± 4.2, 79.3 ± 2.9 and 84.5 ± 4.5%, respectively. Recoveries of fecal daidzein, genistein, and THB were 78.2 ± 5.2, 77.2 ± 4.6 and 80.2 ± 4.4%, respectively. Urinary and fecal isolavones from the feeding study were adjusted according to recoveries for each isolavone, based on the recovery of the internal standard THB.

**Physical activity evaluation.** Energy expenditure (kJ/(kg·d)) was evaluated using the Seven-Day Physical Activity Recall (11). Maximal oxygen uptake (VO2 max) was measured using a continuous, multistage treadmill (Q55; Quinton Instrumentation, Bothell, WA) test following the Bruce protocol (17). The test was stopped when the subject either reached volitional exhaustion or exhibited signs/symptoms warranting test termination. Subjects were considered to have achieved end when VO2 max reached a plateau at the peak workload and the respiratory exchange ratio exceeded unity.

**Statistical analysis.** Statistical analysis was performed with SAS (SAS Institute, version 6.12; 1998, Cary, NC). Descriptive statistics for the dependent and independent variables included means and SEM. A cluster test was conducted to classify isolavone disappearance phenotypes. Pearson correlation analysis was used to determine the correlation between daidzein and genistein disappearance, urinary isolavone excretion and GTT. Multivariate ANOVA and multiple comparison Tukey’s test using general linear models were conducted to determine the differences between isolavone disappearance phenotypes, differences between the two ethnicities and differences between urinary and fecal isolavone excretion. A paired T test was done to verify individual phenotype stability. A χ2 test was used to determine the differences between the two ethnic groups in phenotypic distribution of fecal isolavone disappearance. All results were reported as mean ± SEM. Differences were considered significant at P < 0.05 for all analyses.

**RESULTS**

**Characteristics of ethnic groups.** Caucasian subjects had greater BMI, body fat content, physical activity, VO2 max and intakes of insoluble fiber and dairy foods than did Asian subjects (P < 0.05), whereas Asian subjects had greater red meat intake than did Caucasian subjects (P < 0.05, Table 1). Daily energy intake and total dietary fiber intake did not differ between ethnic groups (Table 1). The frequency of soy food intake was ~1–1.5 times/wk in Asians, which was significantly greater than the 0.5 times/mo in Caucasians.

**Fecal isolavone disappearance phenotype and stability over 5 mo.** Isolavone disappearance by the action of fecal components, presumably gut microorganisms, varied among the subjects (Tables 2 and 3). Based on how rapidly daidzein disappeared from the culture media in phase I, three daidzein disappearance phenotypes were identified in subjects using a cluster test. Daidzein disappearance rate constant Dk < 0.15 h−1 constituted the low disappearance phenotype. Dk between 0.15 and 0.3 h−1 was the moderate disappearance phenotype and Dk > 0.3 h−1 was the high disappearance phenotype. Dk corresponding to each phenotype were significantly different. Based on genistein disappearance in phase I, two genistein disappearance phenotypes, high and low, were found in subjects by using a cluster test. Genistein disappearance rate constant Gk ≤ 0.3 h−1 constituted the low disappearance phenotype; otherwise subjects were assigned to the high disappearance phenotype. Gk differed significantly between high and low disappearance phenotypes. Gk (0.38 ± 0.02) was greater than Dk (0.20 ± 0.01) in all subjects (P < 0.05), and the two were weakly correlated (r = 0.22, P = 0.01).

Within each phenotype, Dk and Gk did not differ between Asian and Caucasian subjects in both phases except Dk of the high disappearance phenotype in phase II (Tables 2 and 3). Across the phenotypes, Dk was greater in Asians (0.23 ± 0.02) than Caucasians (0.16 ± 0.02) (P < 0.05), but not Gk (Asians: 0.41 ± 0.02; Caucasians: 0.35 ± 0.03).

The stability of the fecal isolavone disappearance phenotype was tested (Tables 2 and 3). For daidzein disappearance phenotype based on Dk, no significant differences were found between phase I and phase II in Asian and Caucasian subjects of moderate and low phenotypes. For the high phenotype, comparing phase II with phase I, Dk was greater in Asian subjects; Caucasian subjects had no high daidzein disappearance phenotype (Table 2). For genistein disappearance phenotype, Gk was stable in both high and low phenotypes between the two phases in Asians and Caucasians (Table 3). Twenty-two of 35 Asian subjects and 17 of 31 Caucasian subjects (total 39 out of 66) switched their daidzein disappearance phenotype. The low genistein disappearance phenotype was more stable and moderate daidzein disappearance phenotype less stable in both ethnicities; 11 of 35 Asians and 16 of 31 Caucasians (total 27 out of 66) switched their genistein disappearance phenotype, and the high genistein disappearance phenotype was more stable than was the low genistein disappearance phenotype in both ethnic groups. Individual phenotype stability was verified by paired T test; only Dk between phase I and phase II in Caucasian subjects was different (P < 0.05), corresponding to the absence of the high

**TABLE 1**

<p>| Characteristics of Asian and Caucasian women in phases I and II1 |
|-------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>BMI</th>
<th>Body fat content (%)</th>
<th>VO2 max2</th>
<th>Physical activity</th>
<th>Energy intake (kcal)</th>
<th>Total dietary fiber (g)</th>
<th>Insoluble fiber (g)</th>
<th>Dairy foods</th>
<th>Red meat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>35</td>
<td>20.0 ± 0.7a</td>
<td>26.6 ± 0.9</td>
<td>40.8 ± 1.1a</td>
<td>148.0 ± 3.6a</td>
<td>10.6 ± 28b</td>
<td>26.2 ± 24</td>
<td>1.3 ± 0.5a</td>
<td>1.2 ± 0.2a</td>
<td>0.9 ± 0.1b</td>
</tr>
<tr>
<td>Caucasian</td>
<td>33</td>
<td>24.6 ± 0.8b</td>
<td>29.0 ± 0.9</td>
<td>45.5 ± 1.1bc</td>
<td>164.3 ± 4.0b</td>
<td>8.6 ± 28a</td>
<td>25.6 ± 23</td>
<td>4.0 ± 0.4c</td>
<td>2.8 ± 0.2b</td>
<td>0.3 ± 0.1a</td>
</tr>
<tr>
<td><strong>Phase II</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>35</td>
<td>20.4 ± 0.7a</td>
<td>26.9 ± 0.9</td>
<td>42.6 ± 1.1ab</td>
<td>147.1 ± 4.6a</td>
<td>9.3 ± 28ab</td>
<td>23.4 ± 22</td>
<td>1.6 ± 0.4ab</td>
<td>1.1 ± 0.2a</td>
<td>1.0 ± 0.1b</td>
</tr>
<tr>
<td>Caucasian</td>
<td>31</td>
<td>24.3 ± 0.8b</td>
<td>28.3 ± 1.0</td>
<td>46.9 ± 1.2c</td>
<td>161.8 ± 4.0b</td>
<td>9.0 ± 28ab</td>
<td>24.7 ± 23</td>
<td>3.1 ± 0.5bc</td>
<td>3.0 ± 0.2b</td>
<td>0.4 ± 0.1a</td>
</tr>
</tbody>
</table>

1. Values are means ± SEM. Two Caucasian subjects withdrew from the study in phase II due to graduation and a health problem. Means in a column with superscripts without a common letter differ, P < 0.05.
2. VO2 max, maximal oxygen uptake.
Ethnic differences in phenotypic distribution. The distribution of subjects among daidzein disappearance phenotypes was significantly different between Asian and Caucasian subjects in both phases (Table 2). Compared with Caucasian subjects, more Asian subjects were of the high disappearance phenotype. Caucasian, but not Asian subjects had significantly different distribution among daidzein disappearance phenotypes between phase I and phase II (Table 2). No significant difference was found in the distribution of subjects among genistein disappearance phenotypes between Asians and Caucasians at either time point (Table 3).

Urinary and fecal isoflavone recoveries and GTT. The overall urinary isoflavone excretion was significantly greater than fecal isoflavone excretion in both Asians and Caucasians (P < 0.05, Tables 4 and 5). Overall urinary and fecal daidzein excretion was significantly greater than genistein excretion in both ethnic groups (P < 0.05, Tables 4 and 5). Within and between ethnic groups, urinary and fecal isoflavone excretion did not differ among the three daidzein disappearance phenotypes (Table 4). Between genistein disappearance phenotypes, Asian subjects of the low phenotype had significantly greater urinary genistein (P < 0.05) and marginally greater fecal genistein (P = 0.09) and total urinary isoflavone (P = 0.06) excretion than did Asian subjects of the high phenotype and all Caucasian subjects (Table 5). In Caucasian subjects, no differences were found in isoflavone excretion between high and low genistein disappearance phenotypes. GTT was longer in Caucasians compared with Asians (P < 0.05) (Table 4). GTT did not differ among daidzein disappearance phenotypes within ethnicity (Table 4). Between genistein disappearance phenotypes, Asian subjects of the low phenotype had significantly more rapid GTT than did Asian high phenotype and all Caucasian subjects (Table 5).

Correlation between urinary isoflavone recovery, GTT and isoflavone disappearance rate constant. Total urinary isoflavone excretion was negatively correlated with GTT in all subjects (r = −0.24, P < 0.05). Only Gk but not Dk showed marginally negative correlation with urinary genistein excretion in all subjects (r = −0.20, P = 0.1). In Asian subjects, GTT significantly correlated with Dk (r = 0.35, P < 0.05), whereas in Caucasian subjects, this correlation was marginal (r = 0.28, P = 0.10). Gk was not correlated with GTT in either Asians or Caucasians.

### TABLE 2

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phase I Asian</th>
<th>Phenotype</th>
<th>Phase I Caucasian</th>
<th>Phenotype</th>
<th>Phase II Asian</th>
<th>Phenotype</th>
<th>Phase II Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dk h⁻¹</td>
<td></td>
<td></td>
<td></td>
<td>Dk h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.44 ± 0.02cd</td>
<td>0.47 ± 0.03de</td>
<td>0.54 ± 0.02ce</td>
<td>0</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>0.22 ± 0.02b</td>
<td>0.23 ± 0.01b</td>
<td>0.19 ± 0.02b</td>
<td>9</td>
<td>0.22 ± 0.02b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.06 ± 0.02a</td>
<td>0.08 ± 0.02a</td>
<td>0.07 ± 0.02a</td>
<td>20</td>
<td>0.08 ± 0.01a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All phenotypes</td>
<td>0.25 ± 0.03e</td>
<td>0.20 ± 0.03de</td>
<td>0.21 ± 0.02de</td>
<td>35</td>
<td>0.12 ± 0.03d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM. Two Caucasian subjects dropped out of the study in phase II due to graduation and a health problem.
2 The distribution of subjects among each daidzein disappearance phenotype did not differ between Asians and Caucasians in phase I and phase II (I: $\chi^2 = 6.18$, P < 0.05; II: $\chi^2 = 9.69$, P < 0.05); and between phase I and phase II in Caucasians ($\chi^2 = 8.27$, P < 0.05).
3 Means in a column with superscripts without a common letter (a–c) differ, P < 0.05.
4 Means in a row with superscripts without a common letter (d, e) differ, P < 0.05.
5 ND, not detected.

### TABLE 3

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Phase I Asian</th>
<th>Phenotype</th>
<th>Phase I Caucasian</th>
<th>Phenotype</th>
<th>Phase II Asian</th>
<th>Phenotype</th>
<th>Phase II Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dk h⁻¹</td>
<td></td>
<td></td>
<td></td>
<td>Dk h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0.47 ± 0.02b</td>
<td>0.45 ± 0.03b</td>
<td>0.55 ± 0.03b</td>
<td>16</td>
<td>0.55 ± 0.03b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.19 ± 0.04a</td>
<td>0.18 ± 0.02a</td>
<td>0.14 ± 0.04a</td>
<td>15</td>
<td>0.19 ± 0.02a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All phenotypes</td>
<td>0.39 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.43 ± 0.03</td>
<td>31</td>
<td>0.38 ± 0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM. Two Caucasian subjects dropped the study in phase II due to graduation and a health problem.
2 The distribution of subjects among each genistein disappearance phenotype did not differ between Asians and Caucasians in phase I and phase II (I: $\chi^2 = 2.07$, P > 0.05; II: $\chi^2 = 2.74$, P > 0.05).
3 Means in a row with superscripts without a common letter differ, P < 0.05.
showed that the recoveries of urinary iso-
avonoids were isolated from human feces (5); thus it is likely
fl strains (257, 258, 264 and 265) that cleave the C-ring of
human gut micro-
flora; genistein excretion was especially low
in the inoculated rats (25), indicating that gut microflora are
important to iso-
avone bioavailability.

Our previous work demonstrated a relationship between
fetal iso-
avonol disappearance and iso-
avone bioavailability
(26). Eight men provided fresh fecal samples for in vitro
anaerobic incubation with iso-
avonol and consumed a single
dose of soy iso-
avonol with breakfast (1.2 mg total iso-
avonol/
kg). Three iso-
avonol disappearance phenotypes, high
(high (fl 5), were identi-
fl that cleave flavonoids were isolated from human feces (5); thus it is likely
fl ring cleavage of iso-
avonol also occurs. Germfree rats
excreted more urinary iso-
avonol than did rats inoculated with
human gut microflora; genistein excretion was especially low
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avonol/
kg). Three iso-
avonol disappearance phenotypes, high (n = 1),
moderate (n = 2), and low (n = 5), were identified among the
8 subjects on the basis of the half-lives of iso-
avonol disappear-
ance. Plasma daidzein and genistein concentration was nega-
Negative values were not observed.

DISCUSSION

Due to their involvement in iso-
avonol deconjugation, bio-
transformation and degradation, gut microorganisms may
greatly influence iso-
avonol bioavailability. Iso-
avonol in most
soy foods are primarily β-glucosides. When ingested, iso-
avonol glucosides must undergo hydrolysis of the glucosides to
aglycones before absorption because the glucosides have not
been found in human blood or urine using HPLC or sensitive
electrospray ionization/mass spectrometry (18,19). Iso-
avonol glucoside hydrolysis may occur in the small and large intestine
due to mammalian and bacterial β-glucosidase activity
(19,20). Daidzin was shown to be transformed to daidzein by
human intestinal bacteria Bacteroides J-37 and
Eubacterium
fl (7). After absorption, aglycones conjugate with UDP-
glucuronic acid to form iso-
avonol glucuronides that are rap-
Idly excreted in bile and urine (21). Biliary
excretion allows iso-
avonol to return to the in-
testine to be deconjugated by bacterial glucuronidases. These
aglycones may be reabsorbed, metabolized by bacteria to other
metabolites such as equol and O-demethylangolensin from
daiztein (23,24), or seemingly degraded by gut bacteria be-
cause the recovery of iso-
avonol from feces is a very small
percentage of the ingested dose and < 50% of the ingested
dose is typically recovered from urine (3,18). Four Clostridium
strains (257, 258, 264 and 265) that cleave the C-ring of
flavonoids were isolated from human feces (5); thus it is likely
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important to iso-
avonol bioavailability.

1 Values are mean ± SEM. Two Caucasian subjects dropped the study in phase II due to graduation and a health problem. Means in a column with superscripts without a common letter differ, P < 0.05. * Different from fecal iso-
avonol recovery, P < 0.05.

### TABLE 4

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
<th>Urinary daidzein</th>
<th>Urinary genistein</th>
<th>Fecal daidzein</th>
<th>Fecal genistein</th>
<th>GTT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asian</strong></td>
<td></td>
<td>% ingested dose</td>
<td></td>
<td>% ingested dose</td>
<td></td>
<td>h</td>
</tr>
</tbody>
</table>
| High      | 9 | 14.2 ± 3.7      | 5.7 ± 3.1        | 1.0 ± 0.8     | 0.5 ± 0.6       | 68 ± 9
| Moderate  | 6 | 11.0 ± 4.6      | 4.8 ± 3.8        | 0.5 ± 1.0     | 0.2 ± 0.7       | 56 ± 11
| Low       | 20| 14.2 ± 2.5      | 6.5 ± 2.1        | 2.1 ± 0.6     | 0.8 ± 0.4       | 51 ± 6 |
| **Caucasian** |  |                 |                  |              |                |     |
| High      | 0 | ND              | ND               | ND           | ND             | ND  |
| Moderate  | 9 | 13.5 ± 2.1      | 3.8 ± 0.8        | 1.0 ± 0.2     | 0.1 ± 0.1       | 99 ± 10 |
| Low       | 22| 10.8 ± 1.3      | 3.4 ± 0.5        | 0.7 ± 0.1     | 0.3 ± 0.1       | 77 ± 7  |

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### TABLE 5

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
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<td>% ingested dose</td>
<td></td>
<td>% ingested dose</td>
<td></td>
<td>h</td>
</tr>
<tr>
<td>High</td>
<td>25</td>
<td>11.9 ± 2.1</td>
<td>4.0 ± 1.7</td>
<td>1.3 ± 0.5</td>
<td>0.3 ± 0.3</td>
<td>63 ± 5</td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
<td>18.2 ± 3.4</td>
<td>11.0 ± 2.7</td>
<td>2.0 ± 0.8</td>
<td>1.4 ± 0.5</td>
<td>40 ± 8</td>
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<tr>
<td><strong>Caucasian</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>13.0 ± 1.5</td>
<td>3.6 ± 0.6</td>
<td>0.9 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>85 ± 10</td>
</tr>
<tr>
<td>Low</td>
<td>15</td>
<td>10.2 ± 1.6</td>
<td>3.5 ± 0.6</td>
<td>0.8 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>86 ± 10</td>
</tr>
</tbody>
</table>

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tively correlated with daidzein and genistein disappearance rate constant \((r = -0.74, P = 0.04; r = -0.88, P = 0.01\), respectively), supporting an important role for gut microbial activity in isoflavone bioavailability. In agreement with the previous study, our results showed that Asian subjects of the low genistein disappearance phenotype had greater urinary flavone bioavailability. In agreement with the respective correlations, supporting an important role for gut microbial metabolism of isoflavones and isoflavone bioavailability.

In addition to the isoflavone disappearance phenotype, our results suggested that GTT affects isoflavone bioavailability as well. GTT may be a crucial determinant of the difference in isoflavone bioavailability. We showed that Asian subjects of the low genistein disappearance phenotype with significantly shorter GTT exhibited significantly more urinary genistein than subjects with significantly slower GTT (Asians of the GTT decreased significantly faster than all Caucasians). In Caucasian subjects, there was no significant difference in GTT between high and low genistein disappearance phenotypes, which seemed to prevent the effect of a low disappearance phenotype to increase apparent absorption of genistein. The lack of difference in GTT among daidzein disappearance phenotypes may also explain why we did not find differences in urinary daidzein excretion among daidzein disappearance phenotypes for both Asian and Caucasian subjects. The finding of no differences overall between Asians and Caucasians in isoflavone bioavailability even though the GTT differed between ethnic groups may be due to the induction of microbial isoflavone disappearance in some Asian subjects as a result of frequent soy food intake; there were more Asians than Caucasians who were high degraders of daidzein (Table 2). Lu et al. (27) showed decreased urinary excretion of genistein and daidzein in women but not in men after 1 mo of soymilk feeding providing 80–210 mg/d of each isoflavone, compared with initial isoflavone excretion, suggesting such an adaptation of the gut over time. Gut transit was more rapid in Asians than Caucasians and in Asians of the low genistein disappearance phenotype than in Asians of the high genistein disappearance phenotype. Gut transit may be influenced by diet and physical activity, but neither of these factors accounted for the ethnic difference in GTT. It had been shown that only when insoluble fiber intake was increased to 20 g/d was GTT decreased significantly in healthy adults (28). In this study, Caucasians actually consumed significantly more insoluble fiber than did Asians (by \(\sim 2 \text{ g/d}\)), but overall insoluble fiber intake of subjects was low (Table 1) and unlikely to affect GTT. Increased fitness and physical activity level may affect bowel habits and decrease GTT due to increased colonic motor activity (29). But in one study, 14 healthy sedentary men and women were trained to increase physical fitness. With diet constant, physical fitness was monitored and showed significant improvement after 15 wk of training. However, no change was observed in overall mean daily fecal weight, GTT, bowel movement frequency or dry stool weight (30). This suggested that exercise had no consistent effect on large bowel function and perhaps only extreme differences in physical activity or fitness significantly alter GTT. Even though Caucasian subjects in the present study had a significantly 10% higher level of physical activity and fitness than did Asian subjects (Table 1), they had slower GTT than did Asians.

Gut microorganisms may affect GTT. Bifidobacterium animalis containing dairy products significantly accelerated human GTT (31). We did not identify gut microflora in this study, but such differences might explain significant differences in GTT between ethnicities, given that other factors such as physical activity or dietary habits do not provide plausible explanations.

Genistein disappeared more rapidly than daidzein from the incubation media in both Asians and Caucasians as reflected in fecal disappearance rate constant. This difference may due to the structural differences between daidzein and genistein. It is likely that genistein is more susceptible than daidzein to C-ring cleavage by gut bacteria due to an A-ring 5-hydroxyl (32), thus absent from daidzein. The weak correlation between daidzein and genistein disappearances suggests that isoflavone-metabolism gut microorganisms differ to some extent between the two isoflavones.

We found that daidzein but not genistein disappearance differed significantly between Asians and Caucasians as reflected in daidzein phenotypic distribution and \(D_k\). This might be due to variation in gut microflora caused by ethnic differences in dietary factors, such as red meat and soy food intake. Red meat may affect the species and distribution of intestinal microflora. Peach et al. (33) reported that feces from humans fed a high carbohydrate diet contained a larger proportion of Eubacteria and fewer Bacteroides than did humans fed a high meat diet. Reddy et al. (34) assessed the effects of high meat and meatless diets on the distribution of bacterial species in man. Total anaerobic microflora such as Bacteroides, Bifidobacteria, Peptococci and anaerobic Lactobacilli were present in significantly greater amounts in feces after feeding a high meat diet. Hengtes et al. (35) also examined the effect of a high beef diet on the bacterial content of human feces. Bacteroides and Clostridia counts were significantly greater during a high beef than during a meatless diet. Some of these strains of bacteria may be responsible for hydrolysis of flavonoids and isoflavones from glucosides to aglycones and/or for isoflavone degradation (5). A greater proportion of Asian than Caucasian subjects may have acquired daidzein-degrading organisms because of greater meat consumption. The frequent soy food intake found in Asians may also be responsible for the ethnic differences in daidzein disappearance. Soy food intake may induce bacterial daidzein metabolism and disappearance, but a clear explanation of fecal and presumably gut microbial daidzein disappearance remains to be determined. Dietary fiber intake may also influence gut microbial activity. In this study, however, Caucasian subjects had the same total dietary fiber intake as Asian subjects and only \(\sim 2 \text{ g}\) greater insoluble fiber intake than Asians. This modest difference in insoluble fiber intake seemed unlikely to alter bacterial species distribution or cause ethnic differences in daidzein disappearance.

We did not find an ethnic difference in genistein disappearance phenotypic distribution. This may also be explained by the interindividual differences between daidzein and genistein and the difference between daidzein-metabolism organisms and genistein-metabolism organisms, which in turn influence isoflavone disappearance by gut microflora activities. In both ethnicities, many subjects who were identified within low or moderate daidzein disappearance phenotypes had relatively greater genistein disappearance, thus permitting less distinction among subjects for disappearance of genistein than for daidzein.

The present study showed that relatively rapid GTT coupled with a low fecal genistein disappearance phenotype as occurred in Asian but not Caucasian subjects produced greater genistein bioavailability, as reflected in urinary genistein excretion. This has implications for the design of human isoflavone feeding trials, in that interindividual variability might be lessened and study power increased by selecting subjects based...
upon fecal isoflavone disappearance phenotype and gut transit time. These findings also have implications for the human bioavailability of dietary phenolics in general, which may undergo gut microbial metabolism similar to isoflavones. Human chronic disease susceptibility may depend at least in part on gut transit time and gut microbial ecology due to the potential influence of these factors on the bioavailability of disease preventive phytochemicals.

LITERATURE CITED


