Greater Apparent Absorption of Flavonoids Is Associated with Lesser Human Fecal Flavonoid Disappearance Rates

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Abstract
-OH-flavonoids disappeared more rapidly from human fecal incubations and were less absorbable by humans than flavonoids without 5-OH moieties. Anaerobic fecal disappearance rates over 24 h were determined for 15 flavonoids in samples from 20 men and 13 women. In these anaerobic fecal mixtures, flavonoids with 5,7,40-OH groups, genistein, apigenin, naringenin, luteolin, kaempferol, and quercetin (disappearance rate, k = 0.46 (0.10 h-1), and methoxylated flavonoids, hesperetin and glycitein (k = 0.24 (0.21 h-1), disappeared rapidly compared with flavonoids lacking 5-OH (e.g., daidzein, k = 0.07 (0.03 h-1). Apparent absorption of flavonoids that disappeared rapidly from in vitro fecal incubations, genistein, naringenin, quercetin, and hesperetin, was compared with that of daidzein, a slowly disappearing flavonoid, in 5 men and 5 women. Subjects ingested 104 μmol of genistein and 62 μmol of daidzein (soy milk), 1549 μmol of naringenin and 26 μmol of hesperetin (grapefruit juice), and 381 μmol of quercetin (onions) in three test meals, each separated by 1 week. Blood and urine samples were collected over 24 h after each test meal. Plasma flavonoid concentrations ranged from 0.01 to 1 μM. The apparent absorption, expressed as percentage of ingested dose excreted in urine, was significantly less for naringenin (3.2 (1.7%), genistein (7.2 (4.6%), hesperetin (7.3 (3.2%), and quercetin (5.6 (3.7%) compared with daidzein (43.4 (15.5%, p = 0.02). These data affirmed the hypothesis that the 5,7,40-OH of flavonoids limited apparent absorption of these compounds in humans.

Keywords
Flavonoid; isoflavone; bioavailability; metabolism; human

Disciplines
Food Science | Human and Clinical Nutrition | Molecular, Genetic, and Biochemical Nutrition

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Greater Apparent Absorption of Flavonoids Is Associated with Lesser Human Fecal Flavonoid Disappearance Rates

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It was hypothesized that 5,7,4'-OH flavonoids disappeared more rapidly from human fecal incubations and were less absorbable by humans than flavonoids without 5-OH moieties. Anaerobic fecal disappearance rates over 24 h were determined for 15 flavonoids in samples from 20 men and 13 women. In these anaerobic fecal mixtures, flavonoids with 5,7,4'-OH groups, genistein, apigenin, naringenin, luteolin, kaempferol, and quercetin (disappearance rate, \( k = 0.46 \pm 0.10 \ \text{h}^{-1} \)), and methoxylated flavonoids, hesperetin and glycitein (\( k = 0.24 \pm 0.21 \ \text{h}^{-1} \)), disappeared rapidly compared with flavonoids lacking 5-OH (e.g., daidzein, \( k = 0.07 \pm 0.03 \ \text{h}^{-1} \)). Apparent absorption of flavonoids that disappeared rapidly from in vitro fecal incubations, genistein, naringenin, quercetin, and hesperetin, was compared with that of daidzein, a slowly disappearing flavonoid, in 5 men and 5 women. Subjects ingested 104 \( \mu \text{mol} \) of genistein and 62 \( \mu \text{mol} \) of daidzein (soy milk), 1549 \( \mu \text{mol} \) of naringenin and 26 \( \mu \text{mol} \) of hesperetin (grapefruit juice), and 381 \( \mu \text{mol} \) of quercetin (onions) in three test meals, each separated by 1 week. Blood and urine samples were collected over 24 h after each test meal. Plasma flavonoid concentrations ranged from 0.01 to 1 \( \mu \text{M} \). The apparent absorption, expressed as percentage of ingested dose excreted in urine, was significantly less for naringenin (3.2 \% \pm 1.7 \%), genistein (7.2 \% \pm 4.6 \%), hesperetin (7.3 \% \pm 3.2 \%), and quercetin (5.6 \% \pm 3.7 \%) compared with daidzein (43.4 \% \pm 15.5 \%, \( p = 0.02 \)). These data affirmed the hypothesis that the 5,7,4'-OH of flavonoids limited apparent absorption of these compounds in humans.

KEYWORDS: Flavonoid; isoflavone; bioavailability; metabolism; human

Flavonoids are polyphenolic compounds that are widely distributed in foods of plant origin (1). Daily intakes in humans range from a few milligrams to 1 g (2). Flavonoids in fruits and vegetables have been suggested to lower the risk of steroid-dependent cancers (3). A recent meta-analysis showed that cocoa, tea, and soy flavonoid intake reduced several heart disease risk factors (4) in populations consuming large amounts of flavonoids, but other flavonoids are not well-studied to date. Over 5000 flavonoids have been identified to date and are divided into subclasses, which differ in their heterocyclic C ring (5), including flavones, flavanones, flavonols, and isoflavones (Figure 1). Substitution patterns on the A and B rings with hydroxyl, methyl, methoxyl, O- and C-sugars, acyl, prenyl, sulfate, and glucuronide groups provide additional structural variation in each flavonoid subclass (5).

Flavonoid glycosides are their predominant forms in foods. The glycosides are absorbed to a very limited extent (5) and are cleaved by gut bacterial or human intestinal \( \beta \)-glycosidases (6). Flavonoid aglycons are absorbed across the intestinal mucosa and conjugated in the mucosa and liver by phase II enzymes (UDP-glucuronosyltransferase, sulfotransferase, and catechol-O-methyltransferase) (7). The flavonoids may be excreted in the urine or bile. Bacteria in the lower intestine hydrolyze the flavonoid conjugates after biliary excretion, which results in reabsorption of the flavonoid aglycons and enterohepatic recirculation (7,8). The gut bacteria apparently also further degrade the aglycons (9).

Determination of the metabolism and bioavailability of flavonoids is crucial in the assessment of their health effects, especially knowing the extent of absorption of intact flavonoid aglucons. Apparent absorption and plasma concentrations of isoflavones were strongly related to fecal contents of these compounds. Two women who excreted 10-fold more isoflavones in feces than did five other women also showed different apparent absorption of isoflavones; plasma daidzein and genistein were similar in the two high fecal isoflavone excreters, but daidzein was apparently absorbed to a significantly greater extent than genistein in the low fecal excreters (10). This laboratory has developed in vitro human fecal fermentation systems to determine flavonoid disappearance rate (10), identify fecal isoflavone metabolites, and predict human bioavailability of these compounds (10–13). Slow in vitro fecal disappearance rates of daidzein and genistein corresponded to greater daidzein and genistein bioavailability in human subjects, measured by the mean amount of isoflavones recovered in urine as a percentage of ingested dose (11). The rate

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of fecal isoflavone disappearance in vitro depended on the chemical structure of isoflavones (10, 11). Genistein, which has a 5-OH, was degraded significantly more quickly than daidzein, which does not possess this structural feature (10), and in most subjects, daidzein was significantly more bioavailable than genistein, as reflected in urinary excretion (10, 13).

The chemical structure and substitution pattern of other flavonoids, flavones, flavanones, and flavonols influenced their in vitro anaerobic fecal disappearance rates (14). Although numerous gut microbial metabolites may be formed from flavonoids, most evidence of bioactivity to date has focused on the parent flavonoids. A recent study of flavonoids found predominantly in tea and onions showed that the plasma contents of the various gut microbial flavonoid metabolites were insignificant compared with the parent flavonoid aglycons and suggested that the main biological effects of the flavonoids are probably attributable to the parent compounds (15). In the present study, we hypothesized that the chemical structure of flavonoids determined their rate of fecal disappearance in vitro and their apparent absorption in humans.

**MATERIALS AND METHODS**

**Subjects.** Thirty-three subjects (20 male and 13 female) were recruited from Iowa State University for the in vitro flavonoid degradation study. The subjects’ ages ranged from 18 to 37 years (mean age = 25.6 ± 4.4 years) with a body mass index (BMI) of 18.1–46.1 kg/m² (mean BMI = 23.7 ± 4.9 kg/m²), respectively. The ethnicities of the subjects included 15 Caucasians, 7 Asian Indians, 7 Chinese, 3 African Americans, and 1 African.

Ten (5 male and 5 female) of the 33 subjects participated in the human bioavailability studies. These subjects were selected on the basis of a moderate or low in vitro fecal daidzein disappearance phenotype (average \( k = 0.053 ± 0.029 \) h⁻¹) to attempt to limit interindividual variability in flavonoid bioavailability (13). These subjects were 18–30 years of age (mean age = 25.0 ± 4.0 years) with BMI of 18.1–29.1 kg/m² (mean BMI = 22.6 ± 3.3). The ethnicities included three Caucasians, three Chinese, two Asian Indians, and two African Americans (Table 1). All subjects were healthy and not taking any medication. Approval of the study design was obtained from the Iowa State University Human Subjects Research Committee in 2004. The subjects followed an isolavone-, flavonol-, and flavanone-free diet for 1 week before their respective feedings. All subjects were given oral and written instructions on foods and beverages not to consume during each washout period based on the flavonoid levels reported in the USDA database for the flavonoid content of selected foods (16).

**Reagents and Chemicals.** Apigenin, naringenin, kaempferol, luteolin, quercetin, flavone, chrysin, 7,4'-dihydroxyflavone, 6,4'-dihydroxyflavone, 5,4'-dihydroxyflavone, and 5,3'-dihydroxyflavone were from Indofine Chemical Co., Inc. (Hillsborough, NJ). Daidzein and 2,4',4'-trihydroxydibenzoic (THB) were synthesized using the method of Song et al. (17). Genistein was synthesized according to a modification of the method of Chang et al. (18). HPLC grade acetonitrile,

![Flavonoid structures and numbering system](image_url)

**Figure 1.** Flavonoid structures and numbering system.

**Table 1.** Subject Characteristics

<table>
<thead>
<tr>
<th>subject ID</th>
<th>sex</th>
<th>BMI (kg/m²)</th>
<th>age (years)</th>
<th>gut transit time (h)</th>
<th>in vitro anaerobic fecal daidzein degradation rate, ( k (\text{h}^{-1}) )</th>
<th>ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>F</td>
<td>23.2</td>
<td>22</td>
<td>40 ± 9</td>
<td>0.054</td>
<td>Caucasian</td>
</tr>
<tr>
<td>118</td>
<td>F</td>
<td>18.1</td>
<td>24</td>
<td>53±</td>
<td>0.120</td>
<td>Asian</td>
</tr>
<tr>
<td>119</td>
<td>F</td>
<td>22.7</td>
<td>30</td>
<td>67 ± 20</td>
<td>0.047</td>
<td>Asian</td>
</tr>
<tr>
<td>125</td>
<td>F</td>
<td>29.1</td>
<td>24</td>
<td>50±</td>
<td>0.037</td>
<td>African American</td>
</tr>
<tr>
<td>127</td>
<td>F</td>
<td>22.5</td>
<td>26</td>
<td>104±</td>
<td>0.046</td>
<td>African American</td>
</tr>
<tr>
<td>206</td>
<td>M</td>
<td>26.3</td>
<td>24</td>
<td>39 ± 6</td>
<td>0.017</td>
<td>Caucasian</td>
</tr>
<tr>
<td>212</td>
<td>M</td>
<td>20.7</td>
<td>18</td>
<td>23 ± 16</td>
<td>0.036</td>
<td>Asian Indian</td>
</tr>
<tr>
<td>217</td>
<td>M</td>
<td>20.7</td>
<td>30</td>
<td>23 ± 11</td>
<td>0.064</td>
<td>Asian</td>
</tr>
<tr>
<td>224</td>
<td>M</td>
<td>23.7</td>
<td>25</td>
<td>75±</td>
<td>0.030</td>
<td>Caucasian</td>
</tr>
<tr>
<td>229</td>
<td>M</td>
<td>19.0</td>
<td>27</td>
<td>65 ± 9</td>
<td>0.074</td>
<td>Asian Indian</td>
</tr>
<tr>
<td>female</td>
<td></td>
<td>23.1 ± 3.9</td>
<td>25 ± 3</td>
<td>63 ± 25</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td></td>
<td>22.1 ± 2.9</td>
<td>25 ± 4</td>
<td>45 ± 24</td>
<td>0.044</td>
<td></td>
</tr>
</tbody>
</table>

Overall 22.6 ± 3.3 25 ± 4 54 ± 25 0.053 ± 0.029

* aDropped out after the soy milk feeding period from discomfort due to blood withdrawal. bDropped out during the onion feeding period. cMissing standard deviation values due to difficulty observing the red dye or incomplete collection of samples.
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minimize intersubject variability, subjects were prescreened according to HPLC vial. The sauts daidzein were provided from soy milk, flavanones naringenin and hesperetin were from grapefruit juice, and the flavonol quercetin was from sauted red onions. The ingested amount of soy milk or grapefruit juice was 2 cups, containing 28 mg of genistein and 16 mg of daidzein, or 422 mg of naringenin and 8 mg of hesperetin, respectively (aglucon equivalents). The sauted onions (185 g), which provided 115 mg of quercetin, were fed in a three-egg omelet. The subjects consumed each flavonoid source along

Table 1

moderate and low fecal daidzein degradation phenotypes were recruited to ingest flavonoid test meals (moderate and low fecal daidzein degradation phenotypes were estimated using one-way ANOVA, general linear models. All analyses were performed in duplicate, and all data are reported as mean ± SD. The statistical significance of all analyses was set at α = 0.05.

RESULTS

In Vitro Flavonoid Degradation. Fecal degradation rate differences were analyzed for genistein, apigenin, naringenin, kaempferol, luteolin, quercetin, myricetin, hesperetin, chrysin, flavone, daidzein, glycitein, 5,4'-dihydroxy flavone, 6,4'-dihydroxy flavone, 7,4'-dihydroxy flavone, and 5,3'-dihydroxy flavone (Table 2). The 5,7,4'-trihydroxy flavonoids (genistein, naringenin, apigenin, kaempferol, quercetin, and luteolin) rapidly degraded from the fecal incubations, k = 0.46 ± 0.10 h\(^{-1}\) (< 0.0001), except for the 5,7,4'-trihydroxy flavonoid myricetin (3,5,7,3',4',5'-hexahydroxy flavone), with k = 0.04 ± 0.03 h\(^{-1}\). The methylated flavonoids, hesperetin and glycitein, rapidly degraded with k = 0.24 ± 0.21 and 0.18 ± 0.09 h\(^{-1}\) respectively. All other flavonoids
(chrysin, flavone, daidzein, 5,4'-dihydroxyflavone, 6,4'-dihydroxyflavone, 7,4'-dihydroxyflavone, and 5,3'-dihydroxyflavone) were slowly degraded, \( k \sim 0.05 \pm 0.03 \, h^{-1} \).

**Isoflavone Bioavailability.** After ingestion of soy milk, genistein and daidzein plasma concentrations peaked \( \sim 5 \) h after dosing, with \( t_{1/2} \) of 2.1 and 1.1 h, respectively (Table 3). Peak concentrations were \( 0.7 \pm 0.3 \mu mol/L \) for genistein and \( 1.0 \pm 0.4 \mu mol/L \) for daidzein. Mean apparent absorptions of genistein and daidzein (flavonoid excreted in urine as a percentage of ingested dose) were 7.2 \pm 4.8 and 42.6 \pm 16.0\% respectively, (Table 3). In vitro fecal genistein disappearance rates of the 10 subjects clustered into three significantly different groups (\( p < 0.0001 \)); high \(( k = 1.28 \pm 0.45 \, h^{-1} \), \( n = 3 \)), moderate \(( k = 0.35 \pm 0.01 \, h^{-1} \), \( n = 3 \)), and low \(( k = 0.11 \pm 0.07 \, h^{-1} \), \( n = 4 \), Figure 2A). Urinary genistein excretion in low genistein degraders was 11.5 \pm 4.9\% of ingested dose, significantly greater than urinary genistein excretion in moderate \(( 3.5 \pm 1.6\% \) and high genistein degraders \(( 4.9 \pm 1.2\% \), \( p < 0.05 \), Figure 2B). There was no difference in plasma AUC of genistein across individuals of high, moderate, and low genistein disappearance rates \(( 0.096 \pm 0.014 \, \mu mol \cdot h^{-1} \cdot L^{-1} / \mu mol \) ingested; moderate, \( 0.086 \pm 0.061 \, \mu mol \cdot h^{-1} \cdot L^{-1} / \mu mol \) ingested; and low, \( 0.114 \pm 0.044 \, \mu mol \cdot h^{-1} \cdot L^{-1} / \mu mol \) ingested, \( p > 0.1 \)), nor did plasma genistein AUC differ significantly from plasma AUC of daidzein per micromole ingested (Table 3).

**Flavanone Bioavailability.** After ingestion of grapefruit juice, naringin and hesperetin plasma concentrations peaked at 5.1 and 12 h, respectively, with peak plasma concentrations of 0.3 \pm 0.2 and 0.05 \pm 0.09 \mu mol/L, respectively (Table 3). Plasma half-lives were 5.7 h for naringenin and 3.6 h for hesperetin. The mean urinary excretion as percentage of ingested dose of naringin was 3.2 \pm 1.7\%, and that for hesperetin in males was 7.3 \pm 3.2\%. Hesperetin was not recovered in females (Table 3). In vitro fecal naringenin disappearance rates clustered into three significant groups: high \(( mean \, k = 0.63 \pm 0.20 \, h^{-1} \), \( n = 2 \)), moderate \(( mean \, k = 0.20 \pm 0.01 \, h^{-1} \), \( n = 3 \)), and low \(( mean \, k = 0.05 \pm 0.03 \, h^{-1} \), \( n = 4 \). There was no difference in urinary naringenin excretion or plasma AUC across these three phenotypic groups \( (p > 0.05) \). The in vitro fecal hesperetin disappearance rates did not cluster into significantly different subgroups (data not shown).

**Flavonol Bioavailability.** After the ingestion of cooked red onion, plasma quercetin concentrations peaked at 1.5 h with a half-life of \( \sim 9 \) h. Mean peak plasma concentration was \( 0.8 \pm 0.6 \mu mol/L \), AUC of \( 6.1 \pm 6.1 \mu mol \cdot h^{-1} / L \) (not corrected for \mu mol intake). The pharmacokinetics of quercetin was different from that of the other flavonoids, in that the mean time of peak plasma concentration was 1.5 \pm 1.3 h, and significantly earlier than the other flavonoids with a mean \( t_{\text{max}} \) value of 4.4 \pm 1.2 h \(( p = 0.00064 \)). The mean elimination half-life was 9 h and ranged from 1 to 20 h compared to the half-lives of the other flavonoids, which ranged from 1 to 6 h. Urinary excretion of quercetin was 5.6\% of ingested dose (Table 3). In vitro fecal quercetin disappearance did not sort into distinct clusters (data not shown).

**Overall Flavonoid Bioavailability Comparison.** When flavonoids were compared per micromole ingested, plasma AUC values were not significantly different for genistein and daidzein (Table 3). The mean plasma AUC values for genistein and daidzein were significantly greater than the AUC values for naringenin, quercetin, and hesperetin \(( p = 0.001 \)). Naringenin had the least plasma AUC and apparent absorption, both significantly less than all of the other flavonoids tested \(( p < 0.0001 \), Table 3). Apparent absorptions (urinary excretion) were not significantly different between quercetin, hesperetin, and genistein \((6.6\%, \ p > 0.05) \). The apparent absorption of daidzein was significantly greater than that of the other flavonoids \(( p < 0.0001) \).

### DISCUSSION

The flavonoids that were seemingly more rapidly degraded in vitro fecal incubations from 33 subjects included the isoflavone genistein, the flavones apigenin and luteolin, the flavonols quercetin and kaempferol, and the flavanone naringenin. All of these flavonoids have hydroxyl groups on the 5-, 7-, and 4'-positions of the flavonoid backbone structure (Figure 1). These results confirmed previous results which showed that 5,7,4'-trihydroxyflavonoids were most rapidly degraded by fecal microbes from 11 human subjects compared with flavonoids not having these structural features (14). However, myricetin, which also has hydroxyl groups in the 5-, 7-, and 4'-positions, was not degraded rapidly. The rapidly degraded 5,7,4'-trihydroxyflavonoids in this study possessed three to five hydroxyl groups and were similar in their hydrophobicity when analyzed by HPLC (data not shown). Myricetin has six hydroxyl groups on the flavonoid backbone structure and is much more hydrophilic compared with other 5,7,4'-trihydroxyflavonoids, which may hinder its bacterial degradation. Other flavonoids that were rapidly degraded were the methoxylated isoflavanone and flavanone, glycitein and hesperetin, respectively.

### Table 2. In Vitro Anaerobic Human Fecal Flavonoid Degradation Rates \((n = 33\) Subjects\)

<table>
<thead>
<tr>
<th>flavonoid</th>
<th>in vitro degradation rate ((h^{-1}))</th>
<th>flavonoid</th>
<th>in vitro degradation rate ((h^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>hesperetin</td>
<td>0.75 \pm 0.05a</td>
<td>chrysin</td>
<td>0.08 \pm 0.05c</td>
</tr>
<tr>
<td>naringin</td>
<td>0.47 \pm 0.28b</td>
<td>flavanol</td>
<td>0.08 \pm 0.05c</td>
</tr>
<tr>
<td>genestein</td>
<td>0.38 \pm 0.32b</td>
<td>5,4'-dihydroxyflavone</td>
<td>0.07 \pm 0.04e</td>
</tr>
<tr>
<td>apigenin</td>
<td>0.37 \pm 0.18b</td>
<td>flavone</td>
<td>0.07 \pm 0.02c</td>
</tr>
<tr>
<td>kaempferol</td>
<td>0.35 \pm 0.31b</td>
<td>daidzein</td>
<td>0.07 \pm 0.03c</td>
</tr>
<tr>
<td>luteolin</td>
<td>0.21 \pm 0.16b</td>
<td>7,4'-dihydroxyflavone</td>
<td>0.05 \pm 0.02c</td>
</tr>
<tr>
<td>glycitein</td>
<td>0.18 \pm 0.09bc</td>
<td>6,4'-dihydroxyflavone</td>
<td>0.04 \pm 0.03c</td>
</tr>
<tr>
<td>myricetin</td>
<td>0.12 \pm 0.17b</td>
<td>myricetin</td>
<td>0.04 \pm 0.04c</td>
</tr>
</tbody>
</table>

### Table 3. Bioavailability Parameters of Ingested Flavonoids in Human Subjects

<table>
<thead>
<tr>
<th>isoflavone</th>
<th>N</th>
<th>ingested dose ((\mu mol))</th>
<th>in vitro fecal disappearance rate ((h^{-1}))</th>
<th>relative plasma AUC ((\mu mol \cdot h^{-1} / L \cdot \mu mol ) ingested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>genistein</td>
<td>10</td>
<td>103.6</td>
<td>0.55 \pm 0.56a</td>
<td>0.088 \pm 0.046a</td>
</tr>
<tr>
<td>daidzein</td>
<td>10</td>
<td>61.8</td>
<td>0.05 \pm 0.03b</td>
<td>0.210 \pm 0.060a</td>
</tr>
<tr>
<td>naringenin</td>
<td>9</td>
<td>1549.5</td>
<td>0.23 \pm 0.25a</td>
<td>0.002 \pm 0.001c</td>
</tr>
<tr>
<td>hesperetin</td>
<td>5</td>
<td>25.5</td>
<td>0.75 \pm 0.05a</td>
<td>0.027 \pm 0.035b</td>
</tr>
<tr>
<td>quercetin</td>
<td>8</td>
<td>380.5</td>
<td>0.35 \pm 0.31a</td>
<td>0.016 \pm 0.016b</td>
</tr>
</tbody>
</table>

\( ^{a} \)Letters indicate significant differences, \( p < 0.05 \).

### Notes

- Bioavailability parameter values are means \pm standard deviations.
- Means in a column without a common letter are significantly different, \( p < 0.05 \).
- Disappearance rates measured prior to the feeding studies.
- Hesperetin was not recovered in female subjects.
These methoxylated flavonoids were rapidly demethylated in vitro before further microbial degradation, and their calculated degradation rates were based on the demethylation reaction, and not on disappearance of the demethylation product. In an in vitro human fecal incubation mixture, hesperetin was rapidly demethylated to eriodictyol, which has a 5,7,4'-trihydroxyl flavonoid structure. Eriodictyol then rapidly disappeared, which supported our findings that 5,7,4'-trihydroxyl flavonoids are rapidly degraded (data not shown). Hesperetin was demethylated in an in vitro pig cecum model to eriodictyol and then further degraded to 3-(3-hydroxyphenyl)propionic acid and phloroglucinol (22). We showed that the major degradation pathway of genistein is demethylation to 6,7,4'-trihydroxyl isoflavone (23). Hesperetin disappeared significantly more rapidly than did genistein (Table 2), perhaps as a result of a more rapid demethylation reaction from the 4'-position in hesperetin compared with the 6-position in genistein. The methoxylated isoflavones foromononitin, biochanin A, and glycitein were demethylated regardless of the position of the methoxyl group, but demethylation rates were not reported (24). Perhaps the 5-hydroxyl of hesperetin was responsible for this faster reaction; hesperetin has a 5-hydroxyl group, and glycitein does not.

Because 5,7,4'-trihydroxyl flavonoids disappeared from fecal incubations rapidly in vitro, we tested the hypothesis that the compounds genistein, naringenin, quercetin, and hesperetin would not be very absorbable in humans because these flavonoids would be more likely to be degraded by gut microbes before they could be absorbed, whereas flavonoids such as daidzein lacking one of the 5-, 7-, and 4'-hydroxyl groups would be more absorbable because they disappear at a slower rate and have more time to be absorbed compared with 5,7,4'-trihydroxyl flavonoids.

The apparent absorption of daidzein as reflected in urinary excretion was significantly greater than that for genistein, hesperetin, naringenin, and quercetin (Table 3). The difference in absorption between daidzein and genistein agrees with other studies of subjects ingesting similar doses of genistein and daidzein in soy foods (see, e.g., ref 25). Doses of ~97 μmol of daidzein and 71 μmol of genistein resulted in an average daidzein bioavailability of 19.8%, significantly greater than the mean genistein bioavailability of 5.3% (25).

Apparent absorption of naringenin reflected in urinary excretion was ~3% and significantly less than that of the other flavonoids, hesperetin, genistein, and quercetin, that were rapidly degraded in vitro in fecal samples (Table 2). The plasma AUC of naringenin was also less than that of the other rapidly degraded flavonoids (Table 3). Low naringenin bioavailability was found in previous studies reporting 4–5% apparent absorption (urinary excretion) from a single oral dose of 1837 μmol of pure naringin ingested by human subjects (26). However, a wide range of 5–57% naringenin bioavailability (as urinary excretion) was reported in six subjects ingesting 26 μmol of naringin/kg of body weight (27). Because our study was the first to compare the apparent absorption of these flavonoids, perhaps the far greater dose of naringenin than of the other flavonoids may have limited naringenin uptake. The small number of subjects in our study may have prevented an ability to distinguish effects of interindividual variability in putative gut microbial degradation of naringenin, as seen in degradation rate clusters (see Results, above), on the uptake of this compound.

Most of the available research on hesperetin bioavailability has previously been determined from ingestion of orange juice, because hesperetin is the major flavanone in orange juice, whereas naringenin predominates in grapefruit juice. Hesperetin bioavailability ranged from 3 to 6% (26, 27), which roughly agrees with the mean hesperetin bioavailability of 7% (Table 3). We observed a sex difference in hesperetin bioavailability in that hesperetin was not recovered in the urine or plasma of females, so hesperetin bioavailability was based on the five males (Table 3). The reason for the apparent lack of hesperetin absorption in women is unknown. Perhaps there was sex-specific conversion of hesperetin to a metabolite that was not detectable by our HPLC analyses.

Apparent bioavailability of quercetin was ~6% of ingested dose. These data supported previous data of 6% quercetin bioavailability, t_max = 0.68 h and t_1/2 = 10.9 h, after 12 human subjects ingested 331 μmol of quercetin in onions (28), a dose comparable with our study (381 μmol). Lesser apparent quercetin absorption of 1% was shown after intake of 300 g of lightly fried yellow onions in five subjects (29). The reason for the difference in these bioavailability values is not clear, but may be due to substances in the omelet or in red onions that facilitated the absorption of quercetin.

Plasma AUCs of the flavonoids generally corresponded with urinary excretion, except in the case of genistein. Although plasma AUC of daidzein was >2-fold more than that of genistein when expressed per μmol of quercetin, there was no significant difference in the AUC of genistein and daidzein, perhaps because of great interindividual variability and small numbers of subjects. Because 4 of 10 subjects had low fecal degradation rates for genistein (Figure 2), mean plasma AUC for genistein may be expected to be more similar to that for daidzein, on the basis of a previous study in which plasma daidzein was either greater than or similar to plasma genistein depending on the subset of subjects studied (10). Two women who had ~10-fold greater apparent absorption

Figure 2. In vitro fecal disappearance phenotypes for genistein and correlation with urinary excretion: (A) cluster analysis of genistein in vitro fecal disappearance rates; (B) amount of genistein excreted in urine after 24 h in subjects with high, moderate, and low in vitro fecal genistein disappearance phenotypes (bars with different letters were significantly different, p < 0.05).
of both isoflavones absorbed both isoflavones to a similar extent, whereas five other women who had lesser overall isoflavone absorption showed greater absorption of daidzein than of genistein ($I_0$).

We observed significant interindividual variation within compounds in fecal flavonoid degradation rates in vitro (Table 2). For all of the flavonoids except hesperetin and quercetin, cluster analysis revealed three significant groupings ($p < 0.0001$, data not shown); high, moderate, and low flavonoid disappearance rates. Low flavonoid degraders may experience greater bioavailability of flavonoids compared with high flavonoid degraders because, in the low flavonoid degraders, the flavonoids have more time to be absorbed before they disappear. For example, women ($n = 12$) with low fecal isoflavone disappearance rates experienced greater apparent absorption of isoflavones over 24 h compared with women with high fecal isoflavone disappearance rates ($n = 13$) ($I_2$). Although previous studies indicate that interindividual variability in fecal degradation rates is an important factor in interpreting flavonoid bioavailability data ($I_0$ $-$ $I_2$) (Table 2), the current study showed differences across these fecal degradation rate clusters only for the apparent absorption (urinary excretion) of genistein (Figure 2). A 2-fold greater difference in fecal degradation rate corresponded with $\sim$2-fold less absorption, and greater fecal degradation rate did not further decrease genistein uptake. Although similar differences across fecal degradation rate clusters occurred for naringenin compared with genistein, perhaps the high dose of naringenin prevented the observation of an effect of these fecal degradation rate clusters on naringenin uptake.

These results provide a partial explanation as to why flavonoids with minor differences in their chemical structure may exert different biological effects. Differences in flavonoid chemical structures affect their apparent intestinal microbial degradation and human intestinal uptake, which is a key factor in the biological activity of flavonoids. A recent hamster study supported the importance of relative isoflavone absorption in determining the efficacy of a soy protein diet to lower cholesterol. Only hamsters clustering as high absorbers as reflected in their urinary excretion of isoflavones exhibited significant reduction of total and non-HDL cholesterol compared with casein-fed controls; these absorbers accounted for the observed lowering of cholesterol by the soy protein diet ($I_0$). More data are needed on the relationship of flavonoid chemical structure to biological effects across and within flavonoid subgroups, but, clearly, intestinal microbial metabolism of these compounds must be considered.

**LITERATURE CITED**


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