A Metabolite Profiling Approach to Identify Biomarkers of Flavonoid Intake in Humans1–3

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Abstract

Flavonoids are phytochemicals that are widespread in the human diet. Despite limitations in their bioavailability, experimental and epidemiological data suggest health benefits of flavonoid consumption. Valid biomarkers of flavonoid intake may be useful for estimating exposure in a range of settings. However, to date, few useful flavonoid biomarkers have been identified. In this study, we used a metabolite profiling approach to examine the aromatic and phenolic profile of plasma and urine of healthy men after oral consumption of 200 mg of the pure flavonoids, quercetin, (-)-epicatechin, and epigallocatechin gallate, which represent major flavonoid constituents in the diet. Following enzymatic hydrolysis, 71 aromatic compounds were quantified in plasma and urine at 2 and 5 h, respectively, after flavonoid ingestion. Plasma concentrations of different aromatic compounds ranged widely, from 0.01 to 10 μmol/L, with variation among volunteers. None of the aromatic compounds was significantly elevated in plasma 2 h after consumption of either flavonoid compared with water placebo. This indicates that flavonoid-derived aromatic compounds are not responsible for the acute physiological effects reported within 2 h in previous human intervention studies involving flavonoids or flavonoid-rich food consumption. These effects are more likely due to absorption of the intact flavonoid. Our urine analysis suggested that urinary 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid may be potential biomarkers of quercetin intake and 1,3,5-trimethoxybenzene, 4-O-methylgallic acid, 3-O-methylgallic acid, and gallic acid may be potential markers of epigallocatechin gallate intake. Potential biomarkers of (-)-epicatechin were not identified. These urinary biomarkers may provide an accurate indication of flavonoid exposure.


Introduction

Flavonoids are natural dietary phytochemicals that are widespread in the human diet. They are present in fruits and vegetables, nuts, grains, and beverages such as red wine, tea, and cocoa (1). Both experimental and epidemiological evidence are consistent with a role for dietary flavonoids in the prevention of cardiovascular disease (2,3).

Daily intake of flavonoids such as quercetin and (-)-epicatechin has been estimated at between 20 and 35 mg/d in the form of various glycosides. Due to poor absorption and phase II metabolism, the circulating levels of flavonoids are very low (1). Substantial amounts of unabsorbed flavonoids undergo extensive metabolic transformation in the human gastrointestinal tract, producing a wide range of aromatic and phenolic compounds (4,5). The majority of flavonoid-derived phenolic and aromatic molecules in urine are thought to be initially generated by the action of intestinal bacteria, followed by absorption into the circulation (6). In vitro experiments show that several flavonoids are extensively metabolized by bacteria in the human gastrointestinal tract to form different phenolic acids and aromatic compounds after cleavage of the polyphenolic C ring (4,5,7). It was proposed that these polyphenol-derived phenolic and aromatic compounds may contribute to the health benefits of polyphenol-rich food observed in epidemiological studies (8,9).

There has been interest in the potential use of flavonoids and their metabolites in urine as biomarkers of human dietary flavonoid intake. Total 24-h urinary excretion of quercetin metabolites, such as quercetin-3-O-glucuronide, 3′-O-methylquercetin, quercetin-3′-O-sulfate, and 3′-O-methylquercetin-3′-O-glucuronide, corresponded to only 4.7% of intake when healthy human volunteers consumed onions (10). Urinary excretion of 3-hydroxyphenylpropionic acid and 4-O-methylgallic acid consistently increased in volunteers supplemented...
with grape seed polyphenols (11) and 4-O-methylgallic acid may also be a good urine biomarker for black tea-derived polyphenol intake (12). Urinary isoflavone acid excretion was more loosely related to coffee intake and may be of limited usefulness as a biomarker for coffee-derived polyphenol consumption (12). Valid biomarkers of flavonoid intake may be valuable where estimation of dietary intake is particularly difficult, e.g., in long-term diet intervention studies. These biomarkers are independent of dietary questionnaire information and thus offer better assessment of dietary intake. Most previous studies were performed to establish biomarkers of flavonoid-rich foods rather than individual flavonoids. As bioactivity and bioavailability for each flavonoid are better understood, it becomes more meaningful to establish biomarkers of specific flavonoid intake.

In this study, we aimed to examine, by using a metabolite profiling approach, the aromatic and phenolic profiles of plasma and urine from healthy men, and 2 and 5 h, respectively, after oral consumption of pure quercetin, (-)-epicatechin, or epigallocatechin gallate (EGCG). We also aimed to evaluate whether these aromatic and phenolic metabolites could be used as specific biomarkers for the intake of and exposure to these flavonoids. Relationships of aromatics and phenolics identified as potential biomarkers with bioactivity of the parent flavonoid in vivo were also investigated.

Materials and Methods

Chemicals and reagents. Acetonitrile and bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane were purchased from Sigma-Aldrich. Phenolic and other aromatic compound standards were purchased from Sigma-Aldrich-Fluka, except 4-O-methylgallic acid and 3-O-methylgallic acid were purchased from Exsyraynthesis. d5-Benzolic acid, phenyl-d3 acid, 3-phenyl-d3 propionic acid, t-cinnamic acid (phenyl-d3), and 4-hydroxybenzoic-2,3,5,6-d4 acid were purchased from Sigma-Aldrich, except 4-acetamide containing 1% trimethylchlorosilane were purchased from Acros Organics. 7,8-dihydroxyflavone-d8, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, 3,5-dimethoxycinnamic acid, 3-methoxy-4-hydroxybenzoic acid, 3-hydroxy-4,5,6-trimethoxybenzoic acid, 4-hydroxybenzoic acid, 3-phenyl-d5 propionic acid, 4-hydroxybenzoic-2,3,5,6-d4 acid were purchased from Sigma-Aldrich-Fluka, except 4-acetamide containing 1% trimethylchlorosilane were purchased from Acros Organics. d5-Benzolic acid, phenyl-d3 acid, 3-phenyl-d3 propionic acid, t-cinnamic acid (phenyl-d3), and 4-hydroxybenzoic-2,3,5,6-d4 acid were purchased from Sigma-Aldrich, except 4-acetamide containing 1% trimethylchlorosilane were purchased from Acros Organics. 7,8-dihydroxyflavone-d8, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, 3,5-dimethoxycinnamic acid, 3-methoxy-4-hydroxybenzoic acid, 3-hydroxy-4,5,6-trimethoxybenzoic acid, 4-hydroxybenzoic acid, 3-phenyl-d5 propionic acid, 4-hydroxybenzoic-2,3,5,6-d4 acid were purchased from Sigma-Aldrich, except 4-acetamide containing 1% trimethylchlorosilane were purchased from Acros Organics.

Participants. Twelve healthy men participated in the study. The study was approved by and performed under the guidelines of the Human Ethics Committee of the University of Western Australia and informed consent was obtained from each of the participants before commencement of the study. All participants were healthy with no evidence of chronic disease. None of the participants consumed >20 g alcohol/day or were taking other medications, antioxidants, or vitamin supplements. They were 43.2 ± 4.3 y old (mean ± SEM), weighed 76.8 ± 2.3 kg, and had a BMI of 25.1 ± 0.8 kg/m2.

Experimental design. The aromatic profiles of 3 common dietary flavonoid aglycones, quercetin, (-)-epicatechin, and EGCG (200 mg each), were assessed after ingestion and compared with a placebo treatment (water only). The study design has been described in detail previously (13). The concentrations of aromatic and phenolic compounds in the plasma and urine samples were measured. Plasma was collected 2 h postflavonoid or water intake and compared with a plasma sample taken at baseline (before treatment). Urine was collected during 5 h after consuming the flavonoids or water and compared with a spot urine sample taken before treatment. Urine creatinine was analyzed at the Core Clinical Laboratory at Royal Perth Hospital using a Technicon Axon analyser (Bayer Diagnostics).

GC-MS analyses of aromatic and phenolic compounds. Aromatic compounds were extracted and derivatized according to a previously published method (14). Briefly, the plasma or urine sample (100 μL) was acidified to pH 4.8 by adding 3 mol/L acetate buffer, pH 4.8 (30 mL/L), before incubation with H. pomatia extract (25 mL/L) (Sigma type H3 G8885, contains β-glucuronidase activity and sulfatase activity) for 18 h at 37°C. The sample was acidified with concentrated HCl (final 2% v/v) and internal standards (1 nmol each of d3-benzoic acid, phenyl-d3 acetic acid, 3-phenyl-d3 propionic acid, t-cinnamic acid (phenyl-d3), 4-hydroxybenzoic-2,3,5,6-d4 acid, 2-hydroxy-3-methoxybenzoic acid, and 2,4,5-trimethoxycinnamic acid) were added into the samples. The sample was loaded onto SPE cartridges containing diatomaceous earth (100 mg/100 μL sample) after centrifuging at 8000 × g for 10 min. The aromatics and phenolics were eluted with ethyl acetate (1.8 mL) into a clean glass tube and the organic solvent removed under nitrogen. The dried sample was derivatized with 30 μL bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane in 10 μL acetonitrile for 4 h at 50°C. Derivatized sample (1 μL) was injected into the GC-MS (EI mode) for analysis. Derivatized samples were analyzed by a Hewlett-Packard 5973 mass selective detector interfaced with a Hewlett-Packard 6890 gas chromatograph and equipped with an automatic sampler and a computer workstation, as previously reported (14). The aromatic and phenolic compounds in the samples were identified by comparing retention times and mass ion ratios of standards and quantitated as described previously (14). Each urine sample was extracted and analyzed by GC-MS in duplicate.

Absorption of quercetin, (-)-epicatechin, and epigallocatechin gallate. Quercetin and (-)-epicatechin are present in plasma and urine as glucuronides, sulfates, and in their methylated forms with very small amounts present in the free form (15,16). Absorption of quercetin, (-)-epicatechin, and EGCG was determined by measuring the amounts of free quercetin, 3'-O-methylquercetin, (-)-epicatechin, 3'-O-methyl(-)-epicatechin, and EGCG after the hydrolysis of conjugates in the baseline, 2-h plasma and 5-h urine samples by GC-MS as previously reported (15).

Measurement of nitrite and nitrate. Nitrite and nitrate concentrations in plasma and urine were determined simultaneously using a previously published GC-MS method (17). Briefly, the sample fluid was spiked with internal standards, sodium nitrite-15N (6 ng), and sodium nitrate-15N (40 ng). The spiked sample was derivatized with acetone and pentafluorobenzyl bromide at 50°C for 30 min. After the removal of acetone, the remaining aqueous phase was extracted with toluene and the organic extract (0.5 μL) was analyzed using an Agilent 6890 gas chromatograph coupled to a 5973 mass spectrometer fitted with a cross-linked methyl silicone column (25 m × 0.20 mm, 0.33-mm film thickness, HP5-MS) using negative-ion chemical ionization. Samples (1.0 μL) were injected in the splitless mode and the oven temperature was held at 70°C for 1 min, then increased to 160°C at a rate of 20°C/min, and finally to 280°C at a rate of 30°C/min. Helium (92.5 kPa and flow rate 0.7 mL/min) was used as the carrier and methane as the reagent gas. Authentic standards and labeled standards were quantified using calibration curves obtained from authentic standards and labeled standards.

Statistical analysis of results. Statistical analyses were performed using SAS version 9.0 or SPSS version 11.5. Data are presented as mean ± SEM. The baseline-adjusted, between-group differences were analyzed with random effects models using PROC MIXED (SAS) with Tukey adjustment for multiple comparisons. In these models, participant was treated as the random effect and treatment period and treatment order as the fixed effects. Pearson correlation coefficients between changes in various plasma and urinary variables were calculated. Differences were considered significant at P < 0.05.

Results

Aromatic and phenolic profiles of plasma and urine. All 71 aromatic and phenolic compounds (measured by our GC-MS method) were detected in the participants’ 2-h plasma samples (Supplemental Tables 1 and 2). None of the treatments affected
the concentration of aromatic compounds compared with the water placebo (Supplemental Table 1). Quercetin supplementation increased the urinary excretion of 11 aromatic compounds 5 h after ingestion compared with the water placebo (P < 0.05) (Table 1), but only 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid concentrations were significantly elevated in at least 10 of the 12 participants (Supplemental Table 3). Only the 1,3,5-trimethoxybenzene urinary level was elevated after (+)-epicatechin ingestion compared with the water placebo (P < 0.05) (Table 1). However, this effect occurred in only 9 of the 12 participants (Table 1). Urinary levels of 1,3,5-trimethoxybenzene, 4-O-methylgallic acid, 3-O-methylgallic acid, and gallic acid were augmented in at least 10 of the 12 participants after EGCG ingestion compared with the water placebo (P < 0.05) (Supplemental Table 3).

**Absorption of flavonoids.** The absorption of quercetin and (+)-epicatechin was investigated by measuring the total quercetin and (+)-epicatechin concentrations present in the circulation 2 h after ingestion and the amounts excreted 5 h after ingestion. The total flavonoid concentration was calculated as the sum of the flavonoid and its 3'-O-methyl-derivatives after enzymatic hydrolysis with glucuronidase and sulfatase. As previously reported, acute treatment with quercetin, (+)-epicatechin, or EGCG significantly elevated the plasma total flavonoid concentration in all 12 participants (Fig. 1). The total amounts of quercetin and (+)-epicatechin excreted in urine during the 5-h period were also significantly increased in all participants after acute treatment (Fig. 2). EGCG was not detected in urine using the GC-MS method.

**Relationships of potential biomarkers with flavonoid exposure.** Changes in urinary concentrations of 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid after quercetin supplementation were significantly correlated with changes in plasma total quercetin (Table 2) and urinary total quercetin (Table 2). Changes in urinary concentrations of 1,3,5-trimethoxybenzene, 4-O-methylgallic acid, 3-O-methylgallic acid, and gallic acid after EGCG supplementation correlated significantly with changes in plasma EGCG concentrations (Table 2). A correlation analysis was not conducted for (+)-epicatechin, because we did not identify a potential biomarker of its intake.

**Relationships of potential biomarkers with in vivo bioactivity.** Changes in urinary concentrations of nitrate correlated significantly with each of the 3 aromatic metabolites of quercetin (Table 2). Changes in urinary concentrations of nitrite were not correlated with any of the 3 aromatic metabolites of quercetin (data not shown). We previously used correlations between nitrite and nitrate markers of the in vivo effects of the flavonoids on nitric oxide status (13).

**Discussion**

There have been few reports on the absorption and bioavailability of specific phenolics after consumption of polyphenol-rich foods. Caffeic acid and 4-O-methylgallic acid concentrations increased significantly after consumption of red wine and dealkoholized red wine compared with water or phenol-stripped red wine (18). Chocolate intake increased the urinary excretion of flavonoid-derived phenolic acids in healthy human participants (19). Urinary 4-O-methylgallic acid was suggested as a good biomarker for black tea-derived polyphenol consumption (12) and 3-hydroxyphenylpropionic acid was reported as a major phenolic acid breakdown product of proanthocyanidin in vivo (11). A recent bioavailability study highlighted the potential usefulness of the quantification of (+)-epicatechin, chlorogenic acid, and gallic acid in 24 h urine as specific biomarkers of tea, coffee, and wine intake, respectively (20). Whereas in vivo bioactivity and bioavailability of specific flavonoids are receiving considerable attention, it is also important to identify the phenolic metabolites arising from specific flavonoids. Our results showed that the urinary levels of 11 compounds were significantly greater after consuming quercetin, whereas only 1 compound was significantly greater after (+)-epicatechin and 4 compounds

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**TABLE 1** Aromatic and phenolic compounds that increased significantly in urine of 12 healthy men 5 h after ingestion of specific flavonoids (200 mg)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Compound</th>
<th>Preintake</th>
<th>Postintake</th>
<th>Fold increase</th>
<th>Participants showing increase</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmol/mmol creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>4-Ethylbenzoic acid</td>
<td>94 ± 31 (0, 291)</td>
<td>446 ± 120 (36, 1232)</td>
<td>3.8</td>
<td>83</td>
<td>0.0149</td>
</tr>
<tr>
<td></td>
<td>Benzoic acid</td>
<td>1374 ± 307 (0, 3867)</td>
<td>4042 ± 851 (1220, 11418)</td>
<td>1.9</td>
<td>83</td>
<td>0.0276</td>
</tr>
<tr>
<td></td>
<td>2-Methoxyphenylacetic acid</td>
<td>357 ± 117 (0, 1085)</td>
<td>945 ± 235 (21, 2201)</td>
<td>1.6</td>
<td>67</td>
<td>0.0136</td>
</tr>
<tr>
<td></td>
<td>4-Ethylphenol</td>
<td>409 ± 174 (0, 2271)</td>
<td>854 ± 209 (334, 2908)</td>
<td>1.1</td>
<td>100</td>
<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>3,4-Dihydroxybenzoylpropionic acid</td>
<td>89 ± 16 (0, 167)</td>
<td>200 ± 41(54, 576)</td>
<td>1.2</td>
<td>58</td>
<td>0.0096</td>
</tr>
<tr>
<td></td>
<td>3,4-Dihydroxypropionic acid</td>
<td>174 ± 55 (0, 708)</td>
<td>252 ± 34 (56, 470)</td>
<td>0.5</td>
<td>17</td>
<td>0.0110</td>
</tr>
<tr>
<td></td>
<td>3-Methylgallic acid</td>
<td>41 ± 12 (0, 158)</td>
<td>59 ± 8 (18, 113)</td>
<td>0.4</td>
<td>33</td>
<td>0.0057</td>
</tr>
<tr>
<td></td>
<td>Vanillic acid</td>
<td>596 ± 131 (190, 1624)</td>
<td>895 ± 512 (0, 6441)</td>
<td>0.4</td>
<td>17</td>
<td>0.0367</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid</td>
<td>364 ± 108 (35, 1162)</td>
<td>481 ± 188 (0, 2082)</td>
<td>0.3</td>
<td>25</td>
<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>3-Phenylpropionic acid</td>
<td>142 ± 60 (0, 710)</td>
<td>181 ± 38 (56, 505)</td>
<td>0.3</td>
<td>67</td>
<td>0.0135</td>
</tr>
<tr>
<td>(+)-Epicatechin</td>
<td>1,3,5-Trimethoxybenzene</td>
<td>273 ± 153 (1, 1734)</td>
<td>717 ± 629 (0, 7624)</td>
<td>1.6</td>
<td>75</td>
<td>0.0323</td>
</tr>
<tr>
<td></td>
<td>EGCG</td>
<td>13,5-Trimethoxybenzene 86 ± 56 (2, 687)</td>
<td>2158 ± 152 (6, 1859)</td>
<td>1.5</td>
<td>100</td>
<td>0.0125</td>
</tr>
<tr>
<td></td>
<td>4-O-Methylgallic acid</td>
<td>8 ± 2 (1,33)</td>
<td>17 ± 3 (3,35)</td>
<td>1.1</td>
<td>83</td>
<td>0.0191</td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
<td>3 ± 1 (0, 6)</td>
<td>6 ± 1 (1,13)</td>
<td>1.1</td>
<td>83</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td>3-O-Methylgallic acid</td>
<td>33 ± 7 (3, 90)</td>
<td>65 ± 11 (10, 128)</td>
<td>1</td>
<td>83</td>
<td>0.0222</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (lower limit, upper limit), n = 12.

2 P values were calculated against water treatment after baseline adjustment (mixed model analysis with Tukey’s test).
significantly increased after EGCG intake compared with the water control (Table 1). These results suggest that some of these aromatic and phenolic metabolites could be used as urinary biomarkers of quercetin, (-)-epicatechin, and EGCG intake.

In our study, an aromatic compound was considered a potential biomarker of the specific flavonoid intake when the urinary level significantly increased (by at least 1-fold between pre- and post-treatment) compared with the water placebo and the increase was observed in at least 80% of the cases (i.e. 10 of 12 participants). Significant increases in urinary excretion of 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid occurred in at least 10 of the 12 participants after quercetin ingestion. The changes in plasma and urinary total quercetin were significantly correlated with those in urinary concentrations of 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid. These results suggest that these compounds are potential candidates for biomarkers of quercetin intake. Furthermore, the changes in urinary concentrations of 4-ethylphenol, benzoic acid, and 4-ethylbenzoic acid were significantly correlated with changes in urinary nitrate concentration, which is an in vivo marker of nitric oxide production. Because plasma concentrations of aromatics were not significantly elevated, this probably reflects the significant effect of quercetin to augment nitric oxide production in these participants (13).

Similarly, 1,3,5-trimethoxybenzene, 4-O-methylgallic acid, 3-O-methylgallic acid, and gallic acid may be possible biomarkers of EGCG ingestion, because their urinary levels were significantly increased in at least 10 participants and changes in their urinary concentrations and plasma EGCG concentration were significantly correlated. EGCG was present at much lower concentrations (0.16 ± 0.01 μmol/L) in the circulation than quercetin (3.54 ± 1.57 μmol/L) and (-)-epicatechin (3.57 ± 1.21 μmol/L) after acute treatment. Similar circulating concentrations of EGCG were reported in a recent study after the ingestion of 300 mg EGCG (21). We carried out experiments to ascertain if EGCG had degraded during the process of dissolution and found that it does degrade with time (up to 45% in 30 min) in the aqueous mixture prepared for this study (200 mg in 300 mL water). However, at least 95% of the prescribed 200-mg dose was present in the aqueous mixture at the time of consumption (1–2 min after dissolution) (data not shown). A previous study has shown that gallic acid and its methylated metabolites may be potential markers of black tea consumption (22). It appears that 1,3,5-trimethoxybenzene may offer a better indication of EGCG intake than 3-O-methylgallic acid and 4-O-methylgallic acid, because the gallic acid metabolites may also be formed from the metabolism of gallic acid in black tea. Our study did not identify any potential biomarker of (-)-epicatechin intake, because 1,3,5-trimethoxybenzene increased in only 8 of 12 participants. The limitations of our data are the acute exposure to flavonoids and a 5-h urine collection. Our results may also lack specificity, because only 3 flavonoids were studied and compared in this experiment. Although these represent major dietary flavonoids, there are still many other flavonoids to be considered.

The participants in our study consumed a flavonoid-restricted diet (avoiding fruits, vegetables, and beverages rich in flavonoids) for 48 h prior to each treatment. Thus, phenolic and aromatic compounds from other dietary sources, together with contributions from a variety of endogenous aromatic metabolic...
TABLE 2  Pearson correlations between changes in plasma and urinary concentrations of flavonoids, aromatic metabolites, and nitrate in 12 healthy men after ingestion of specific flavonoids (200 mg)

<table>
<thead>
<tr>
<th>Change in plasma concentration, μmol/L</th>
<th>Change in urinary concentration, nmol/mmol creatinine</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>4-Ethylphenol</td>
<td>0.946</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Benzoic acid</td>
<td>0.874</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>4-Ethylbenzoic acid</td>
<td>0.932</td>
<td>0.0001</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>1,3,5-Trimethoxybenzene</td>
<td>0.875</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>4-O-Methylgallic acid</td>
<td>0.916</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>3-O-Methylgallic acid</td>
<td>0.884</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
<td>0.818</td>
<td>0.050</td>
</tr>
</tbody>
</table>

In conclusion, in this study we examined the acute effects of specific flavonoid intake on the in vivo profile of aromatic and phenolic compounds. Our results also identify some specific urinary metabolites that may be potential acute biomarkers of quercetin and EGCG intake. These results are particularly interesting because of the potential health benefits of these dietary flavonoids and the need to accurately measure their intake. Quantification of urinary phenolic compound profiles may provide an additional measure of flavonoid intake along with the conventional food questionnaire.

Acknowledgments

W.M.L. carried out most of the experimental work and wrote the first draft of the manuscript; A.M.J. developed the GC-MS methods and supervised the analysis; J.M.P. carried out and supervised some of the experimental work and revised the manuscript; A.J.M. helped supervise some of the experimental work and revised the manuscript; J.M.H. supervised statistical analysis of data and helped revise the manuscript; B.H. helped develop and supervise the GC-MS analysis and revised the manuscript; K.D.C. had overall supervision of the project and final revision of the manuscript. All authors read and approved the final version.

Literature Cited


