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# Metabolic Profiling of Flavonol Metabolites in Human Urine by Liquid Chromatography and Tandem Mass Spectrometry

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Twenty-one flavonol metabolites have been identified by LC/ESI-MS/MS in human urine, including isomers, after the consumption of cooked onions. Metabolites identified include quercetin monoglucuronides, methyl quercetin monoglucuronides, a quercetin monoglucuronide sulfate, quercetin diglucuronides, a methyl quercetin diglucuronide, quercetin glucoside sulfates, methyl quercetin, quercetin, and kaempferol monoglucuronides. The fragmentation patterns of flavonol metabolites obtained by MS/MS were distinctive for some isomers, indicating that fragmentation patterns may be useful predictors of conjugation position. Two isomers of sulfate quercetin glucosides were also found in urine, suggesting that many of the quercetin glucosides in onion are absorbed intact and undergo metabolism to the sulfate conjugate. Additionally, the interindividual variation in urinary quercetin metabolite profiles was determined by comparing the relative level of six different quercetin metabolites excreted in the urine of healthy volunteers. The ranges of quercetin metabolites excreted were similar among volunteers, yet notable differences in the levels of metabolites among individuals were observed. This study demonstrates the potential of monitoring the range of quercetin metabolites to reveal information on interindividual biotransformation capacity in response to dietary manipulations and as a biomarker for flavonol consumption.

**KEYWORDS: Flavonoids; flavonol; quercetin; kaempferol; metabolite; metabolism; tandem mass spectrometry; bioavailability; human; elimination**

## INTRODUCTION

Flavonoids are potent antioxidants that occur in most plant species and can account for a significant percentage of the chemical constituents in vegetables, fruits, and beverages such as tea and red wine (*1*, *2*). Contemporary interest in flavonoids focuses on their potent antioxidant properties and the epidemiological association between flavonol-rich diets and a lower incidence of cardiovascular disease (*3*) and certain cancers (*4*, *5*). Flavonoids are diphenylpropanes with different oxidation levels that are generally subclassified on the basis of the oxidation state of the central C ring (e.g., flavones, flavonols, flavanones, flavanols, anthocyanidins, and isoflavones). The major flavonols found in the Western diet are glucosides of quercetin (**Figure 1**). Major dietary sources of quercetin glycosides are onions (∼200−650 mg/kg), apples (∼36 mg/kg), and broccoli (∼6−30 mg/kg) (*1*, *6*, *7*).

The bioavailability and metabolism of flavonols such as quercetin are complex. In general, ∼75−99% of ingested quercetin glycosides are not recovered in urine, and levels of the quercetin aglycons in human plasma rarely exceed 1 $\mu$M when the quantities ingested do not exceed those common in the diet (*8*). The small intestine is the primary site of absorption of quercetin glycosides (*8*−*11*), and it appears that the sugar moiety of the glycoside is an important determinant in the absorption process (*12*, *13*). The absorption of quercetin glucosides is thought to occur via interactions with epithelial brush border membrane transporters, such as the sodium-dependent glucose transporter-1 (SGLT-1), followed by deglycosylation (*12*, *14*, *15*). Additional studies indicate that flavonoids may also be absorbed after deglycosylation by hydrolases (e.g., lactose-phlorizin hydrolase or $\beta$-glucosidase) located at the intestinal brush border membrane (*16*−*18*). In either case, once the flavonoid is absorbed, it is further metabolized by UDP-glucuronyl transferases, sulfotransferases, and methyl transferase, forming numerous glucuronidated,

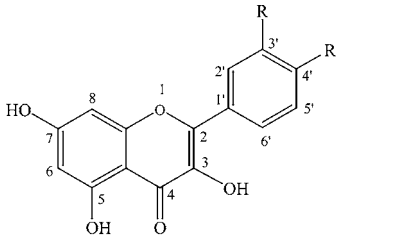


**Figure 1.** Representative structures of quercetin and kaempferol.

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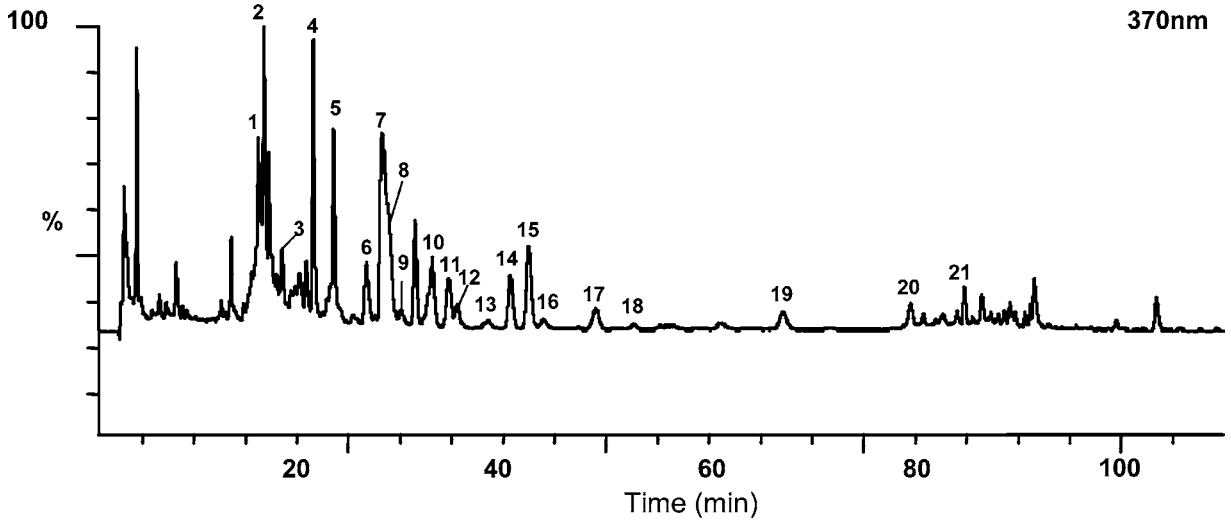


**Figure 2.** Representative HPLC chromatogram of human urine 4 h after the consumption of 200 g of cooked onions. Numbered peaks represent quercetin metabolites identified by LC/ESI-MS/MS in positive ion mode.

sulfated, and methylated conjugates prior to reaching systemic circulation (*19*−*22*).

To date, a range of quercetin metabolites have been described, and accumulating evidence indicates that many of these metabolites have biological activity. However, there remains an increasing need to establish the physiological function and mechanism of action of individual metabolites and determine their distribution in tissues. In studies of rats fed quercetin 4′-*O*-glucoside, 10 quercetin metabolites were identified in plasma, whereas 16 metabolites were identified in the intestine (*22*). In humans fed onions, quercetin monoglucuronides, methyl quercetin glucuronide, and a quercetin sulfate conjugate were identified in plasma (*21*). Circulating glucuronides, sulfate, and *O*-methylated forms of flavonols are believed to be those most likely to exert bioactivities and express beneficial effects in humans and animals (*23*−*26*). In in vitro assays measuring the inhibition of xanthine oxidase and lipoxygenase, Day et al. demonstrated that all quercetin glucuronides inhibited xanthine oxidase and lipoxygenase, with the exception of quercetin 3-glucuronide, and that this activity is dependent upon the conjugation position (*27*). Shirai et al. also showed that quercetin 3′-*O*-glucuronide significantly inhibited lipid peroxidation in liposomal membranes, although its inhibitory effect was lower than that of quercetin aglycon (*27*). Studies such as these demonstrate the need to further investigate both qualitatively and quantitatively the range of flavonoid metabolites in biological tissues.

In the current study we describe the use of LC/ESI-MS/MS employing selected ion monitoring (SIM) to examine an extensive range of quercetin and kaempferol (**Figure 1**) metabolites in human urine after the consumption of onion, a food rich in these flavonols. Additionally, we explore the use of urinary metabolite profiles to describe interindividual differences in quercetin metabolism and biotransformation capacity.

## MATERIALS AND METHODS

**Chemicals.** All HPLC solvents were purchased from Fisher (Pittsburgh, PA). Other reagents and chemicals were purchased from either Fisher or EM Science (Gibbstown, NJ) as analytical grades.

**Study Design.** Six subjects (five women and one man) between 20 and 40 years of age, weighing between 48 and 65 kg, volunteered for this study. None of the subjects were pregnant, lactating, or had any chronic illness. All subjects were nonsmokers. Subjects fasted for 12 h prior to the consumption of 200 g of cooked onions, to avoid contributions of other food flavonoids. Onions were sliced and lightly sauted in a minimal amount of corn oil for 10 min.

**Sample Pretreatment.** Urine (between 80 and 100 mL) was collected in a sterile container at 2 and 4 h postconsumption from each individual. Aqueous methanol (1:1, v/v) was added to the urine samples, and the samples were centrifuged at 4000 rpm at room temperature for 20 min. The supernatant was collected and subjected to rotary evaporation at 45 °C to remove methanol. The samples were then lyophilized. A 25 mg aliquot of the freeze-dried sample was dissolved in 100 $\mu$L of water and filtered through a 0.45 $\mu$m PTFE, HPLC membrane filter prior to LC-MS analysis. This sample was diluted with methanol (1:1, v/v). The injection volume was 10 $\mu$L.

**LC/ESI-MS/MS Analysis.** Metabolites were separated by a reversed phase HPLC system (Shimadzu Scientific, Columbia, MD) equipped with an SIL-10A autoinjector, binary LC 10AD pumps, and an SPD-10A UV−vis detector monitoring at 370 nm. Metabolites were separated on a 250 × 2 mm i.d., 5 $\mu$m, Prodigy, ODS column (Phenomenex, Torrance, CA). The mobile phase consisted of 1% formic acid in water (A) and 1% formic acid in acetonitrile (B). Separations were effected by a series of linear gradients using a flow rate of 0.2 mL/min as follows: elution starting with 5% B, 0−10 min; 5−20% B, 10−30 min; 20% B, 30−50 min; 20−21% B, 50−70 min; 21% B, 70−80 min; 21−60% B, 80−90 min; 60% B, 90−95 min; 60−100% B, 95−100 min. Column eluent was directed into a Quattro LC triple-quadrupole mass spectrometer (Micromass, Altrincham, U.K.) equipped with a dual orthogonal (*ZSPRAY*) ion source. Samples were run in positive ion mode solution using a capillary voltage of 3.2 kV. The cone and extractor voltages were set to 20 and 2 V, respectively. The source temperature and a desolvation gas temperature were 150 and 300 °C, respectively. Optimum nebulization was achieved using a nitrogen flow rate of 800 L/h. Total ion chromatograms (TIC) were recorded over a mass range of *m*/*z* 50−800. The *m*/*z* values for all peaks in the TIC were determined to identify all possible quercetin metabolites in these samples. This approach allows for the preliminary identification of metabolites that may not have been realized and is critical to the success of selected ion monitoring (SIM). Peaks showing *m*/*z* values corresponding to possible quercetin metabolites were further investigated using electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). Parent ions were detected by SIM, and daughter ions were generated using data-dependent scanning techniques. Data were collected and processed using MassLynx software (v 3.5).

## RESULTS AND DISCUSSION

Urine samples were collected at 2 and 4 h after the consumption of 200 g of cooked onion, equivalent to ∼53.8 mg of quercetin (*6*), from six volunteers, and 4 h urine samples were chosen for analysis. **Figure 2** shows the representative

**Table 1.** Quercetin and Kaempferol Metabolites Identified in Human Urine

| peak | compound | $[M + H]^+$ (*m*/*z*) | major fragment ions (*m*/*z*) |
|---|---|---|---|
| 1 | quercetin diglucuronide | 655 | 479 ($[M + H]^+$ – GlucUA[a]), 303 ($[M + H]^+$ – GlucUA – GlucUA) |
| 2 | methyl quercetin diglucuronide | 669 | 493 ($[M + H]^+$ – GlucUA), 317 ($[M + H]^+$ – GlucUA – GlucUA) |
| 3 | quercetin diglucuronide | 655 | 479 ($[M + H]^+$ – GlucUA), 303 ($[M + H]^+$ – GlucUA – GlucUA) |
| 4 | quercetin diglucuronide | 655 | 479 ($[M + H]^+$ – GlucUA), 303 ($[M + H]^+$ – GlucUA – GlucUA) |
| 5 | quercetin monoglucuronide | 479 | 303 ($[M + H]^+$ – GlucUA) |
| 6 | quercetin glucoside sulfate | 545 | 383 ($[M + H]^+$ – Glc[a]), 303 ($[M + H]^+$ – Glc – $SO_3$) |
| 7 | quercetin monoglucuronide sulfate | 559 | 383 ($[M + H]^+$ – GlucUA), 303 ($[M + H]^+$ – GlucUA – $SO_3$) |
| 8 | quercetin glucoside sulfate | 545 | 383 ($[M + H]^+$ – Glc), 303 ($[M + H]^+$ – Glc – $SO_3$) |
| 9 | kaempferol monoglucuronide | 463 | 287 ($[M + H]^+$ – GlucUA) |
| 10 | methyl quercetin monoglucuronide | 493 | 317($[M + H]^+$ – GlucUA) |
| 11 | quercetin monoglucuronide | 479 | 303 ($[M + H]^+$ – GlucUA) |
| 12 | methyl quercetin monoglucuronide | 493 | 317 ($[M + H]^+$ – GlucUA) |
| 13 | methyl quercetin monoglucuronide | 493 | 317 ($[M + H]^+$ – GlucUA) |
| 14 | quercetin monoglucuronide | 479 | 303 ($[M + H]^+$ – GlucUA) |
| 15 | methyl quercetin monoglucuronide | 493 | 317 ($[M + H]^+$ – GlucUA) |
| 16 | kaempferol monoglucuronide | 463 | 287 ($[M + H]^+$ – GlucUA) |
| 17 | methyl quercetin monoglucuronide | 493 | 317 ($[M + H]^+$ – GlucUA) |
| 18 | kaempferol monoglucuronide | 463 | 287 ($[M + H]^+$ – GlucUA) |
| 19 | kaempferol monoglucuronide | 463 | 287 ($[M + H]^+$ – GlucUA) |
| 20 | quercetin | 303 | 303 ($[M + H]^+$) |
| 21 | methyl quercetin | 317 | 317 ($[M + H]^+$) |

[a] GlucUA, glucuronic acid; Glc, glucose.

UV chromatogram collected at 370 nm ($\lambda_{max}$ for quercetin) at 4 h. This HPLC chromatogram indicated that there were 20−30 peaks representing possible quercetin metabolites in urine. In preliminary studies we investigated the rate of elimination of quercetin by monitoring the appearance of quercetin glucuronides and sulfated and methylated conjugates by LC/ESI-MS/MS at 2, 4, 6, and 8 h postconsumption of 200 g of cooked onion. The urine collected at 4 h presented the greatest abundance and range of quercetin metabolites (data not shown), although the elimination kinetics of the various metabolites differed among individuals. On the basis of these findings we chose the 4 h time point for comparisons of quercetin metabolite profiles among subjects. In other studies, Hollman et al. (*9*) reported that the peak plasma level of quercetin in human was reached 2.9 h after the ingestion of onion and that the half-life of the elimination phase was 16.9 h. Aziz et al. (*28*) showed the peak plasma levels of quercetin 4′-glucoside after onion consumption were reached after ∼1.3 h.

Quercetin metabolites were separated by reversed phase HPLC monitoring at 370 nm. Additionally, TICs were collected by full scanning ions over a mass range of *m*/*z* 50−800. This range encompasses the *m*/*z* values of all possible quercetin metabolites. The *m*/*z* values for each of the peaks appearing in the UV profile (**Figure 2**) were extracted from the TIC. Peaks showing *m*/*z* values corresponding to possible quercetin metabolites were further investigated using positive ion mode electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). Parent ion-to-daughter ion transitions of all peaks were determined, and the major daughter ion transitions of peaks identified as quercetin metabolites are described in **Table 1**. Identified metabolites include the aglycon quercetin (peak 20 in **Figure 2**; $[M + H]^+$ at *m*/*z* 303) together with a combination of methylated, sulfated, and glucuronide derivatives of quercetin.

Three quercetin diglucuronides were identified by monitoring ions corresponding to the $[M + H]^+$ of quercetin diglucuronides at *m*/*z* 655 (**Figure 3A**). These peaks correspond to peaks 1, 3, and 4 in **Figure 2**. Parent ion-to-daughter ion transitions of peaks a−c (**Figure 3A**, a−c) give rise to the quercetin monoglucuronide ($[M + H]^+$ at *m*/*z* 479) and the quercetin aglycon ($[M + H]^+$ at *m*/*z* 303), indicating that these peaks are quercetin diglucuronides. The fragmentation patterns of peaks a−c (**Figure 3A**) differed in the composition and intensities of minor ions and in their LC retention times, indicating that they represent different isomeric forms that arise from variation in the positioning of the glucuronyl moiety on the flavonol ring. Further studies employing NMR are needed to understand how MS/MS fragmentation patterns relate to isomeric forms of quercetin metabolites. Peak 2 in **Figure 2** had an $[M + H]^+$ at *m*/*z* 669, 14 mass units higher than the quercetin diglucuronides, identifying it as a methylated derivative of quercetin diglucuronide. Parent ion-to-daughter ion transitions of this peak gave rise to the methylated quercetin monoglucuronide ($[M + H]^+$ at *m*/*z* 493) as well as methylated quercetin ($[M + H]^+$ at *m*/*z* 317).

Three quercetin monoglucuronides (peaks 5, 11, and 14 in **Figure 2**) were identified by monitoring ions corresponding to the $[M + H]^+$ of quercetin monoglucuronides at *m*/*z* 479. MS/MS spectra (**Figure 3B**, a−c) produced fragment ions corresponding to the quercetin aglycon ($[M + H]^+$ at *m*/*z* 303). Fragments characteristic of quercetin were also observed at *m*/*z* 257, 229, and 153 for some of these peaks (*20*, *29*). The fragment with *m*/*z* 153 is considered to originate from a retro-Diels−Alder fission, resulting in the cleavage of the heterocyclic ring of quercetin (*30*). Again, the fragmentation patterns of these peaks were different, as were their LC retention times, indicating that these are different isomeric forms that arise from variation in the positioning of the glucuronyl moiety on the flavonol ring. Several studies have focused on the identification of quercetin glucuronides. For example, Day et al. (*31*) demonstrated four quercetin monoglucuronide isomers that occur at the four hydroxyl groups (3-, 7-, 3′-, 4′-) using human liver cell-free extracts, whereas Oliveira and Watson (*32*) detected four monoglucuronides after incubation of quercetin with human UGT-1A9 microsomes.

One sulfated form of the quercetin monoglucuronide (peak 7 in **Figure 2**) was also identified by monitoring ions corresponding to $[M + H]^+$ of the quercetin monoglucuronide sulfate at *m*/*z* 559. MS/MS spectra indicated that this peak produces fragment ions corresponding to the quercetin sulfate (*m*/*z* 383 due to loss of a glucuronyl unit) and the quercetin aglycon at *m*/*z* 303. In addition, five methylated forms of quercetin monoglucuronides (peaks 10, 12, 13, 15, and 17 in **Figure 2**)

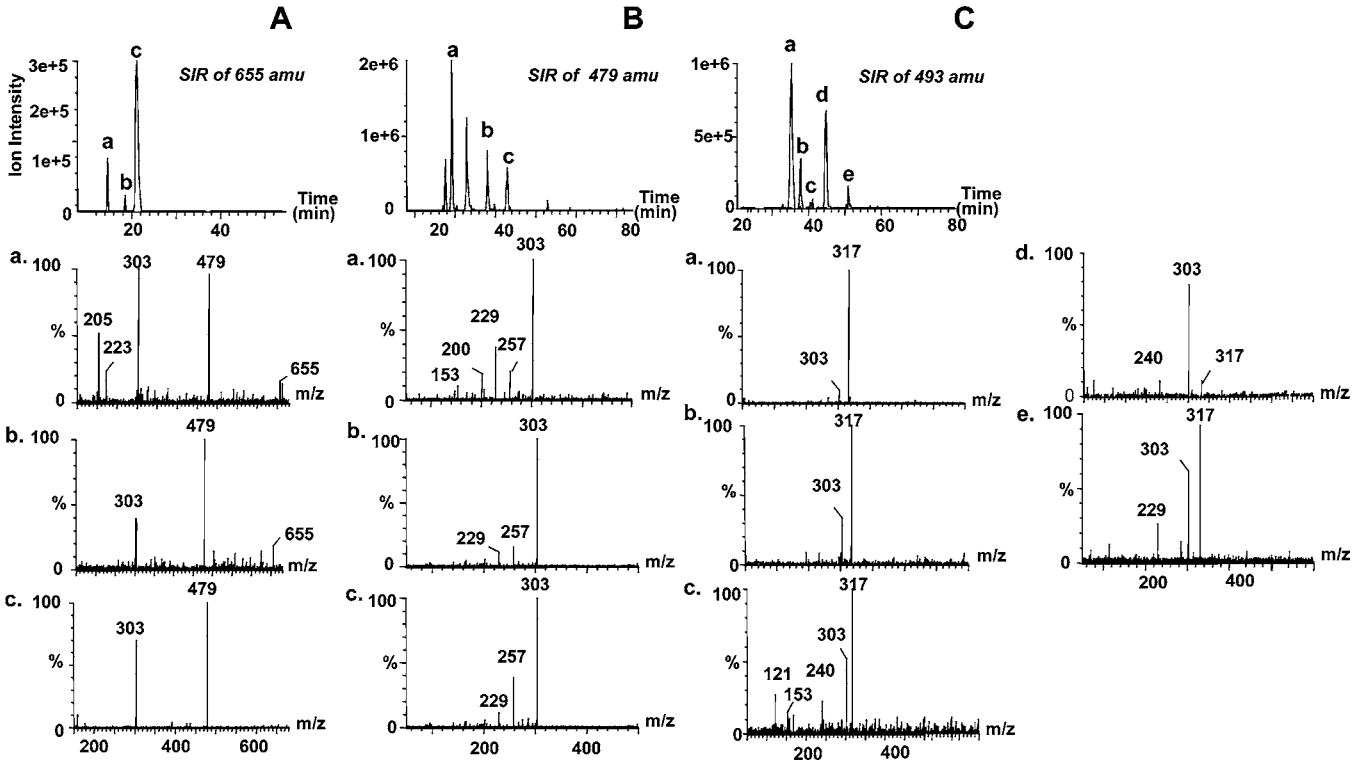


**Figure 3.** Selected ion recording (SIR) and MS/MS spectra of (**A**) quercetin diglucuronide at *m*/*z* 655, (**B**) quercetin monoglucuronide at *m*/*z* 479, and (**C**) methyl quercetin monoglucuronide at *m*/*z* 493 identified in human urine.

were identified by monitoring ions corresponding to the $[M + H]^+$ of methyl quercetin monoglucuronide at *m*/*z* 493. Parent ion-to-daughter ion transitions of peaks a−e (**Figure 3C**, a−e) demonstrate that they give rise to methyl quercetin (*m*/*z* 317 due to loss of the glucuronyl unit) and the quercetin aglycon (*m*/*z* 303). MS/MS spectra again demonstrated that the relative abundance of key fragments ions (e.g., *m*/*z* 317 and 303) varies depending on types of isomer present and may provide information on the position of conjugation. Additionally, a methylated form of quercetin was identified by monitoring ions corresponding to the $[M + H]^+$ of methyl quercetin at *m*/*z* 317. This conjugate corresponds to peak 21 in **Figure 2**.

Interestingly, two quercetin glucoside sulfates (peaks 6 and 8 in **Figure 2**) were identified in this study monitoring $[M + H]^+$ ions at *m*/*z* 545 that correspond to quercetin glucoside sulfates. Three peaks appear in the selected ion chromatograms (**Figure 4A**) that correspond to ions of mass of *m*/*z* 545; however, only two of these peaks (**Figure 4A**, a and b) gave fragment ions corresponding to sulfated quercetin (*m*/*z* 383) and the quercetin aglycon (*m*/*z* 303). To date, there is still considerable debate as to whether quercetin is absorbed as the glycoside or if hydrolysis is requisite for absorption. A number of studies support the view that quercetin glycosides do not reach systemic circulation (*10*, *11*, *33*, *34*). Conversely, other studies demonstrate the presence of nanogram levels of flavonoid glycosides in plasma (*28*, *35*−*37*). For example, Aziz et al. (*28*) reported that isorhamnetin-4′-*O*-*β*-glucoside and quercetin-4′-*O*-*β*-glucoside accumulated in plasma after the consumption of onion. Mauri et al. (*36*) also found the flavonol glycoside rutin (quercetin-3-*O*-rutinoside) in the plasma of healthy volunteers after the consumption of tomato extracts. Rutin is one of the predominant flavonol glycosides in tomatoes. Additionally, Oliveira et al. (*37*) tentatively identified two flavonol glycosides in human plasma after the consumption of capsules of *Ginkgo biloba*, a plant rich in flavonoid glycosides. Our results agree with these latter findings and suggest that quercetin glycosides in onions are absorbed intact and undergo metabolism to the sulfate conjugate.

In total, seven different quercetin metabolites were identified in urine by LC/ESI-MS/MS, including quercetin monoglucuronides, methyl quercetin monoglucuronides, quercetin glucuronide sulfate, quercetin diglucuronides, methyl quercetin diglucuronides, quercetin glucoside sulfate, and methyl quercetin. When isomeric forms are taken into consideration, 16 quercetin metabolites were identified (**Table 1**). In addition, four isomers of kaempferol monoglucuronides were identified (peaks 9, 16, 18, and 19 in **Figure 2**). The selected ion chromatogram (**Figure 4B**) and MS/MS spectra (**Figure 4B**, a−d) indicate that each of these peaks has an $[M + H]^+$ at *m*/*z* 463 corresponding to the kaempferol monoglucuronide and produces a fragment corresponding to kaempferol aglycon (*m*/*z* 287). Kaempferol is the second most abundant flavonoid in onions with concentrations of 0.89 mg/100 g of raw onion (*38*). Volunteers consumed ∼0.70 mg of kaempferol in the 200 g of cooked onions (0.35 mg/100 g of cooked onion) (*38*).

The ranges of flavonoid metabolites found in the urine of all volunteers were remarkably similar. **Figure 5** shows the UV chromatograms taken from the six healthy volunteers 4 h after the consumption of onions. Although these studies are not quantitative, they were rigorously controlled and offer qualitative comparisons. Notable differences in the profile of metabolites formed between subjects were apparent (e.g., compare volunteer 6 with volunteer 5) and demonstrate the potential of monitoring the range of quercetin metabolites to reveal information on interindividual biotransformation capacity.

Urinary excretion of flavonoids such as quercetin has been proposed as a potential biomarker for the intake of fruits and vegetables. This has been tested successfully in studies supplementing the human diet with high-flavonoid foods (*39*, *40*) or with isolated compounds (*10*), showing the positive correlations

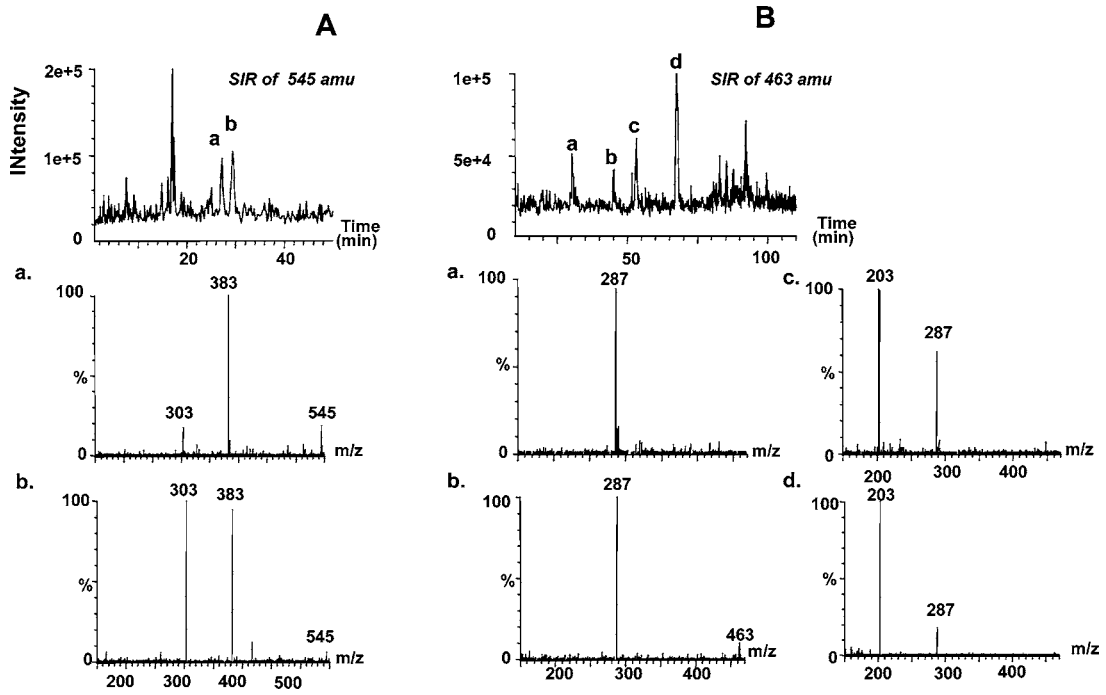


**Figure 4.** Selected ion recording (SIR) and MS/MS spectra of (**A**) quercetin glucoside sulfate at *m*/*z* 545 and (**B**) kaempferol monoglucuronide at *m*/*z* 463 identified in human urine.

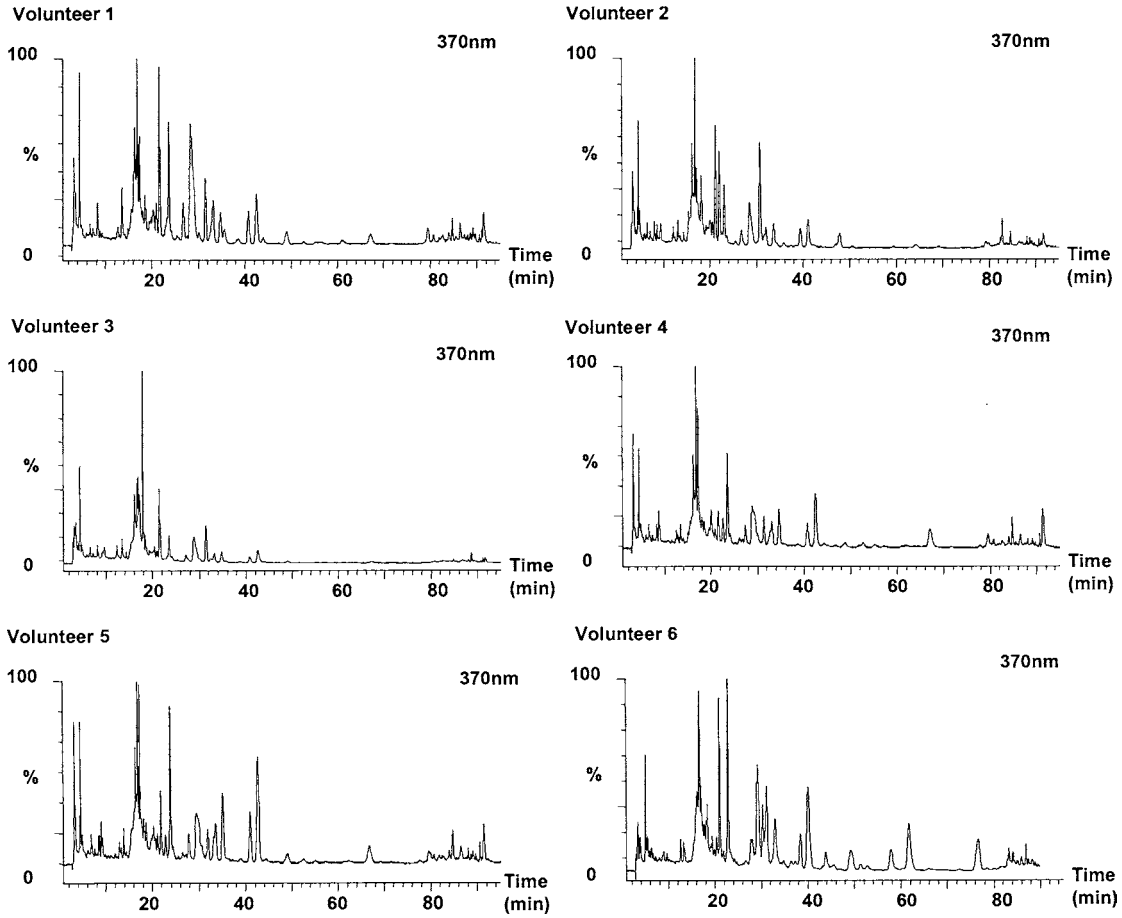


**Figure 5.** HPLC chromatograms of urine from six volunteers collected 4 h after the consumption of 200 g of cooked onions.

between the urinary or plasma level of specific flavonoids (e.g., naringenin, quercetin, and hesperetin), obtained after hydrolysis, and the intake of fruits and vegetables. Krogholm et al. (*41*) demonstrated a linear response in the excretion of flavonoids by comparing the urinary excretion of seven flavonoids in a basic diet, a low-flavonoid diet, and a high-flavonoid diet in

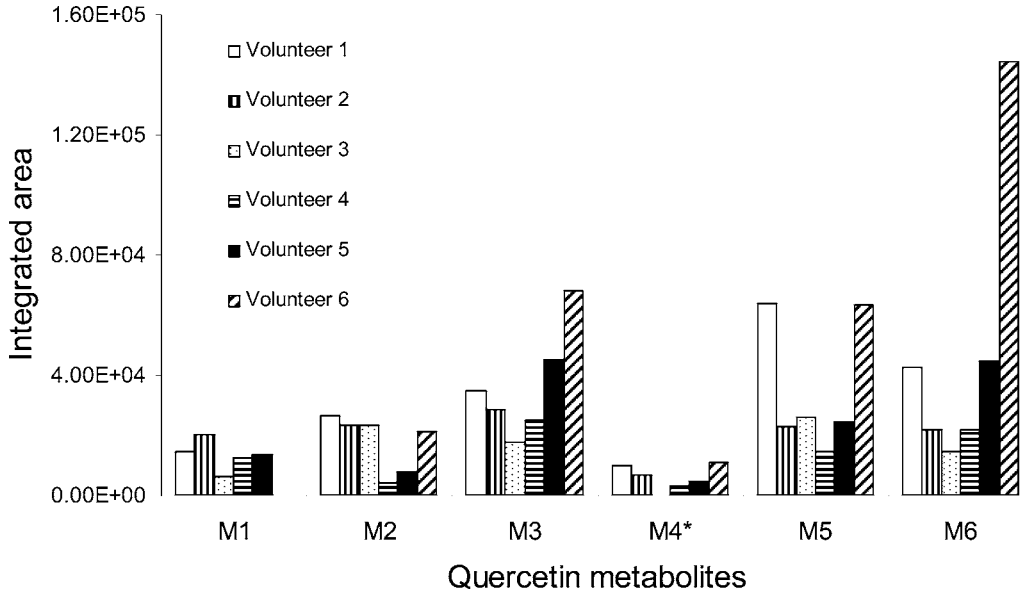


**Figure 6.** Relative comparison of the levels of six quercetin metabolites in urine from six healthy volunteers: M1, methyl quercetin diglucuronide; M2, quercetin diglucuronide; M3, quercetin monoglucoside sulfate; M4, combined peak area of quercetin monoglucoside sulfate and quercetin monoglucuronide sulfate; M5, quercetin monoglucuronide; M6, methyl quercetin monoglucuronide. * For volunteer 4, this value is reported as the combined peak area of one isomer of quercetin monoglucoside sulfate and quercetin monoglucuronide sulfate (both metabolites) as these were not resolved completely during HPLC.

24 h urine. Conversely, other studies indicate that due to the high interindividual variation, measuring total flavonoids is not a particularly precise biomarker for the intake of fruits and vegetables (*41*−*44*). As a way to begin to understand interindividual variation, the relative levels of six primary quercetin metabolites were compared among the volunteers (**Figure 6**). Levels of metabolites were obtained by combining the peak area for all isomers of a specific class of metabolite (e.g., combining the peak areas for all three quercetin monoglucuronides, etc.). One isomer of the quercetin monoglucoside sulfate was not resolved from the quercetin glucuronide sulfate, and therefore the areas of these two peaks were combined for comparisons (**Figure 6**, M4). The metabolites compared in these studies include methyl quercetin diglucuronide, quercetin diglucuronides, quercetin monoglucuronides, one isomer of quercetin monoglucoside sulfate, quercetin monoglucoside sulfate/quercetin monoglucuronide sulfate, and methyl quercetin monoglucuronides. These results demonstrate that the ranges of metabolites excreted are similar among volunteers, yet the levels of metabolites differ among individuals. All subjects excreted diglucuronides, monoglucoside sulfate, monoglucuronides, and methyl monoglucuronide conjugates of quercetin (**Figure 6**, M2, M3, M5, and M6). Methyl quercetin monoglucuronides (**Figure 6**, M6) and quercetin monoglucuronides (**Figure 6**, M5) predominate followed by one quercetin monoglucoside sulfate (**Figure 6**, M3; **Figure 2**, peak 6) and quercetin diglucuronides (**Figure 6**, M2). Volunteer 6 excreted comparatively high levels of methyl quercetin monoglucuronides and was the only subject that did not excrete the methyl quercetin diglucuronide (**Figure 6**, M1). Volunteer 3 (**Figure 5**) did not excrete detectable amounts of the resolved isomer of the quercetin glucuronide sulfate and glucoside (**Figure 6**, M4).

Relative comparisons of quercetin metabolites excreted in the urine of the six volunteers indicate that the ranges of metabolites excreted were similar among volunteers. However, notable differences in the levels of metabolites among individuals were observed. It is well established that certain dietary flavonoids can modulate levels of biotransformation enzymes such as UDP-glucuronosyltransferase, monooxygenase, and glutathione *S*-transferases (*45*−*47*). This study demonstrates the potential of monitoring the range of quercetin metabolites to reveal information on interindividual biotransformation capacity and probe an individual's response to dietary manipulations.

## LITERATURE CITED

(1) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.

(2) Hertog, M. G. L.; Hollman, P. C. H.; Van De Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **1993**, *41*, 1242−1246.

(3) Steinberg, F. M.; Bearden, M. M.; Keen, C. L. Cocoa and chocolate flavonoids: implications for cardiovascular health. *J. Am. Diet. Assoc.* **2003**, *103*, 215−223.

(4) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

(5) Galati, G.; Teng, S.; Moridani, M. Y.; Chan, T. S.; O'Brien, P. J. Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. *Drug Metab. Drug Interact.* **2000**, *17*, 311−349.

(6) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.

(7) Justesen U.; Knethsen P.; T., L. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *J. Chromatogr.* **1998**, *799*, 101−110.

(8) Hollman, P. C.; van Trijp, J. M.; Buysman, M. N.; van der Gaag, M. S.; Mengelers, M. J.; de Vries, J. H.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152−156.

(9) Hollman, P. C.; vd Gaag, M.; Mengelers, M. J.; van Trijp, J. M.; de Vries, J. H.; Katan, M. B. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. *Free Radical Biol. Med.* **1996**, *21*, 703−707.

(10) Erlund, I.; Kosonen, T.; Alfthan, G.; Maenpaa, J.; Perttunen, K.; Kenraali, J.; Parantainen, J.; Aro, A. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur. J. Clin. Pharmacol.* **2000**, *56*, 545−553.

(11) Graefe, E. U.; Wittig, J.; Mueller, S.; Riethling, A.-K.; Uehleke, B.; Drewelow, B.; Pforte, H.; Jacobasch, G.; Derendorf, H.; Veit, M. Pharmacokinetics and bioavailability of quercetin glycosides in humans. *J. Clin. Pharmacol.* **2001**, *41*, 492−499.

(12) Hollman, P. C.; Bijsman, M. N.; van Gameren, Y.; Cnossen, E. P.; de Vries, J. H.; Katan, M. B. The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. *Free Radical Res.* **1999**, *31*, 569−573.

(13) Hollman, P. C.; de Vries, J. H.; van Leeuwen, S. D.; Mengelers, M. J.; Katan, M. B. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr.* **1995**, *62*, 1276−1282.

(14) Wolffram, S.; Block, M.; Ader, P. Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. *J. Nutr.* **2002**, *132*, 630−635.

(15) Ader, P.; Block, M.; Pietzsch, S.; Wolffram, S. Interaction of quercetin glucosides with the intestinal sodium/glucose co-transporter (SGLT-1). *Cancer Lett.* **2001**, *162*, 175−180.

(16) Day, A. J.; DuPont, M. S.; Ridley, S.; Rhodes, M.; Rhodes, M. J.; Morgan, M. R.; Williamson, G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett.* **1998**, *436*, 71−75.

(17) Day, A. J.; Canada, F. J.; Diaz, J. C.; Kroon, P. A.; McLauchlan, R.; Faulds, C. B.; Plumb, G. W.; Morgan, M. R.; Williamson, G. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Lett.* **2000**, *468*, 166−170.

(18) Nemeth, K.; Plumb, G. W.; Berrin, J. G.; Juge, N.; Jacob, R.; Naim, H. Y.; Williamson, G.; Swallow, D. M.; Kroon, P. A. Deglycosylation by small intestinal epithelial cell $\beta$-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur. J. Nutr.* **2003**, *42*, 29−42.

(19) Moon, J. H.; Tsushida, T.; Nakahara, K.; Terao, J. Identification of quercetin 3-*O*-$\beta$-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radical Biol. Med.* **2001**, *30*, 1274−1285.

(20) Wittig, J.; Herderich, M.; Graefe, E. U.; Veit, M. Identification of quercetin glucuronides in human plasma by high-performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B: Biomed. Sci. Appl.* **2001**, *753*, 237−243.

(21) Day, A. J.; Mellon, F.; Barron, D.; Sarrazin, G.; Morgan, M. R.; Williamson, G. Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. *Free Radical Res.* **2001**, *35*, 941−952.

(22) Mullen, W.; Graf, B. A.; Caldwell, S. T.; Hartley, R. C.; Duthie, G. G.; Edwards, C. A.; Lean, M. E.; Crozier, A. Determination of flavonol metabolites in plasma and tissues of rats by HPLC-radiocounting and tandem mass spectrometry following oral ingestion of [2-(14)C]quercetin-4′-glucoside. *J. Agric. Food Chem.* **2002**, *50*, 6902−6909.

(23) Spencer, J. P.; Kuhnle, G. G.; Williams, R. J.; Rice-Evans, C. Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem. J.* **2003**, *372*, 173−181.

(24) Shirai, M.; Yamanishi, R.; Moon, J. H.; Murota, K.; Terao, J. Effect of quercetin and its conjugated metabolite on the hydrogen peroxide-induced intracellular production of reactive oxygen species in mouse fibroblasts. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 1015−1021.

(25) Terao, J. Dietary flavonoids as antioxidants in vivo: conjugated metabolites of (−)-epicatechin and quercetin participate in antioxidative defense in blood plasma. *J. Med. Invest.* **1999**, *46*, 159−168.

(26) Morand, C.; Crespy, V.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. Plasma metabolites of quercetin and their antioxidant properties. *Am. J. Physiol.* **1998**, *275*, R212−219.

(27) Shirai, M.; Moon, J. H.; Tsushida, T.; Terao, J. Inhibitory effect of a quercetin metabolite, quercetin 3-*O*-$\beta$-D-glucuronide, on lipid peroxidation in liposomal membranes. *J. Agric. Food Chem.* **2001**, *49*, 5602−5608.

(28) Aziz, A. A.; Edwards, C. A.; Lean, M. E.; Crozier, A. Absorption and excretion of conjugated flavonols, including quercetin-4′-*O*-$\beta$-glucoside and isorhamnetin-4′-*O*-$\beta$-glucoside by human volunteers after the consumption of onions. *Free Radical Res.* **1998**, *29*, 257−269.

(29) Meng, X.; Maliakal, P.; Lu, H.; Lee, M. J.; Yang, C. S. Urinary and plasma levels of resveratrol and quercetin in humans, mice, and rats after ingestion of pure compounds and grape juice. *J. Agric. Food Chem.* **2004**, *52*, 935−942.

(30) Stevens, J. F.; Taylor, A. W.; Nickerson, G. B.; Ivancic, M.; Henning, J.; Haunold, A.; Deinzer, M. L. Prenylflavonoid variation in *Humulus lupulus*: distribution and taxonomic significance of xanthogalenol and 4′-*O*-methylxanthohumol. *Phytochemistry* **2000**, *53*, 759−775.

(31) Day, A. J.; Bao, Y.; Morgan, M. R.; Williamson, G. Conjugation position of quercetin glucuronides and effect on biological activity. *Free Radical Biol. Med.* **2000**, *29*, 1234−1243.

(32) Oliveira, E. J.; Watson, D. G. In vitro glucuronidation of kaempferol and quercetin by human UGT-1A9 microsomes. *FEBS Lett.* **2000**, *471*, 1−6.

(33) Moon, J. H.; Nakata, R.; Oshima, S.; Inakuma, T.; Terao, J. Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2000**, *279*, R461−467.

(34) Sesink, A. L.; O'Leary, K. A.; Hollman, P. C. Quercetin glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4′-glucoside. *J. Nutr.* **2001**, *131*, 1938−1941.

(35) Paganga, G.; Rice-Evans, C. A. The identification of flavonoids as glycosides in human plasma. *FEBS Lett.* **1997**, *401*, 78−82.

(36) Mauri, P. L.; Iemoli, L.; Gardana, C.; Riso, P.; Simonetti, P.; Porrini, M.; Pietta, P. G. Liquid chromatography/electrospray ionization mass spectrometric characterization of flavonol glycosides in tomato extracts and human plasma. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 924−931.

(37) Oliveira, E. J.; Watson, D. G.; Grant, M. H. Metabolism of quercetin and kaempferol by rat hepatocytes and the identification of flavonoid glycosides in human plasma. *Xenobiotica* **2002**, *32*, 279−287.

(38) USDA database for the flavonoid content of selected foods. March 2003, http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html.

(39) Noroozi, M.; Burns, J.; Crozier, A.; Kelly, I. E.; Lean, M. E. Prediction of dietary flavonol consumption from fasting plasma concentration or urinary excretion. *Eur. J. Clin. Nutr.* **2000**, *54*, 143−149.

(40) de Vries, J. H.; Hollman, P. C.; Meyboom, S.; Buysman, M. N.; Zock, P. L.; van Staveren, W. A.; Katan, M. B. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. *Am. J. Clin. Nutr.* **1998**, *68*, 60−65.

(41) Krogholm, K. S.; Haraldsdottir, J.; Knuthsen, P.; Rasmussen, S. E. Urinary total flavonoid excretion but not 4-pyridoxic acid or potassium can be used as a biomarker for the intake of fruits and vegetables. *J. Nutr.* **2004**, *134*, 445−451.

(42) Erlund, I.; Meririnne, E.; Alfthan, G.; Aro, A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. *J. Nutr.* **2001**, *131*, 235−241.

(43) Erlund, I.; Silaste, M. L.; Alfthan, G.; Rantala, M.; Kesaniemi, Y. A.; Aro, A. Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. *Eur. J. Clin. Nutr.* **2002**, *56*, 891−898.

(44) Brevik, A.; Rasmussen, S. E.; Drevon, C. A.; Andersen, L. F. Urinary excretion of flavonoids reflects even small changes in the dietary intake of fruits and vegetables. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 843−849.

(45) Walle, T.; Otake, Y.; Galijatovic, A.; Ritter, J. K.; Walle, U. K. Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in the human hepatoma cell line hep G2. *Drug Metab. Dispos.* **2000**, *28*, 1077−1082.

(46) Siess, M. H.; Guillermic, M.; Le Bon, A. M.; Suschetet, M. Induction of monooxygenase and transferase activities in rat by dietary administration of flavonoids. *Xenobiotica* **1989**, *19*, 1379−1386.

(47) Debersac, P.; Heydel, J. M.; Amiot, M. J.; Goudonnet, H.; Artur, Y.; Suschetet, M.; Siess, M. H. Induction of cytochrome P450 and/or detoxication enzymes by various extracts of rosemary: description of specific patterns. *Food Chem. Toxicol.* **2001**, *39*, 907−918.

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