Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake

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ABSTRACT Flavonols are antioxidants that may reduce the risk of heart disease. Two major flavonols in the diet are quercetin and kaempferol, and their main sources in the Netherlands are tea and onions. We investigated whether plasma concentrations and urinary excretion of quercetin and kaempferol in humans could be used as biomarkers of intake. We provided 15 subjects with strong black tea (1600 mL/d) or fried onions (129 g/d) for 3 d each in random order separated by a 4-d washout period. The tea provided 49 mg quercetin and 27 mg kaempferol daily and the onions provided 13 mg quercetin and no kaempferol. Flavonols from both foods were clearly absorbed. However, the excretion of unmodified quercetin was 0.5% of intake after tea and 1.1% after onions. Thus, the absorption of quercetin from tea was half that from onions. The onion treatment was repeated 7–14 d later to estimate within-subject CVs as a measure of reproducibility when the same treatment is given twice. CVs for quercetin were 30% in plasma and 42% in urine. The magnitude of these variations relative to actual variations of ≈60% between free-living subjects indicates that concentrations of quercetin in plasma and urine are applicable as biomarkers of its intake. We conclude that flavonols in plasma and urine reflect short-term flavonol intake and that they could be used as biomarkers to distinguish between high and low flavonol consumption in epidemiologic studies. Am J Clin Nutr 1998;68:60–5.

KEY WORDS Quercetin, kaempferol, flavonols, flavonoids, dietary assessment, excretion, tea, onions, reproducibility, biomarker

INTRODUCTION Flavonoids, a group of polyphenolic compounds, are widely present in vegetables, fruit, tea, and wine (1, 2). A main subgroup of the flavonoids are the flavonols, of which quercetin and kaempferol are the major representatives (3, 4). Onions are an important source of quercetin in the diet, and tea is a source of both quercetin and kaempferol (1, 2).

Beneficial effects of flavonols in the diet on the risk of heart disease have been suggested by epidemiologic studies (5, 6), but whether these components can prevent heart disease is still controversial (7). Protective effects of flavonols against cancer have also been proposed (8), but epidemiologic data hitherto fail to support this (9, 10).

Further studies investigating the relation of flavonols to disease are needed. Such studies require an accurate method to assess intake of flavonols in free-living subjects. However, it is difficult to assess flavonol intake by common dietary record or recall methods. Such methods rely on self-report and their accuracy is therefore uncertain (11, 12). It is also difficult to assess true daily flavonol intake because flavonols are concentrated in a few foods (13, 14). Therefore, small errors in the assessment of the consumption of these few flavonol-rich foods result in large errors in estimated flavonol intake. Also, when record or recall methods are used, the intake of flavonols has to be calculated from the flavonol content of foods. The flavonol content of many foods is not known and flavonols are not commonly included in nutrient databases. Moreover, these methods do not take into account the bioavailability of flavonols, which varies widely between foods (15). For example, the percentage of quercetin absorbed from apples was only one-third of that from onions (16). For many foods, such as tea, absorption has not even been reported yet. The use of biomarkers of intake can bypass most of these problems.

Like other dietary assessment methods, biomarkers in population studies suffer from measurement error. This error consists of random and systematic errors (17). Random error includes within-subject fluctuations, metabolic between-subject differences, and imprecision in laboratory analyses. Random error can be determined by studying the reproducibility of the concentration of the marker at constant rates of intake. To determine this reproducibility the same treatment has to be given twice to the same individual. Systematic errors cannot be determined easily, but this error is probably much smaller for biological measurements than for record or recall methods.

The objective of our study was to determine whether concentrations of flavonols in plasma and urine may be used as bio-
markers of their intake. A second objective was to compare the bioavailability of flavonols from tea and onions. Therefore, we measured concentrations of quercetin and kaempferol in plasma and excretion of these flavonols in urine after feeding volunteers tea or onions, and we repeated the treatment with onions to determine reproducibility.

SUBJECTS AND METHODS

Subjects

We obtained approval for the protocol from the Ethics Committee of the Division of Human Nutrition and Epidemiology. We recruited 8 men and 7 women via posters and local newspapers and informed them about the aim of the study and possible discomforts that it could entail. The subjects gave their written informed consent. They were evaluated medically and considered by a physician to be healthy. The mean age of the subjects was 27.6 y (range: 19–56 y) and their mean body mass index (in kg/m²) was 23.5 (range: 19.7–30.9).

Design

The experiment lasted 3 wk. The 3 treatments were provided in random order; each treatment was given for 3 d on days 5–7 of each week. Days 1–4 served as a washout period. The tea treatment consisted of 1600 mL concentrated black tea and the onion treatment of 129 g fried onions, both divided over the day. The onion treatment was given twice to assess reproducibility within subjects.

On days 4–7 of each treatment week the subjects followed a low-quercetin diet. For this purpose they were given a list of vegetables and fruit containing > 15 mg quercetin/kg and beverages containing > 4 mg quercetin/L (1, 2) and were instructed not to consume any of these.

On day 7 of each treatment week blood was sampled and urine was collected for 24 h. On the morning of day 4 of the second week of treatment, blood was sampled to determine baseline values for quercetin in plasma. At this time point the subjects had followed the diet low in quercetin for 1 d and they had not yet started to consume the second treatment. For the baseline value of kaempferol in plasma the concentration after consumption of onions was used as a surrogate because onions do not contain kaempferol (1). Eight months after the experiment we repeated 1 treatment week in 8 of the 15 subjects without providing tea or onions to collect baseline values for urine.

During all treatment weeks subjects recorded all deviations from the guidelines in a diary. Medications were not allowed on any of the treatments and we repeated the treatment with onions to check compliance with the low-quercetin diet by 24-h recalls. Quercetin and kaempferol intakes were calculated by using our published values for contents in vegetables, fruit, and beverages (1, 2).

Collection of blood and urine samples

Venous blood samples were taken between 1200 and 1300 as described previously (19). Urine samples were collected by each subject in 500- and 1000-mL bottles that contained thymol dissolved in isopropanol as a preservative. The first urine voiding after rising in the morning was discarded and all subsequent voids until the next morning, including the first void after rising, were collected. The urine bottles were immediately put into polystyrene boxes containing dry ice. Within 1–5 d the urine samples were thawed at 40°C and pooled by subject and day, and aliquots were stored at −40°C until analyzed. Completeness of urine collection was checked by assessment of recovery in urine of 2.0 mg Li (235 mol) as lithium chloride dissolved in 10 mL water taken by the subjects every morning. On days 5, 6, and 7 they took this under our supervision. The dose of 2 mg Li/d is 1% of that considered safe for chronic use in patients with bipolar disorders (20). The recovery in urine of orally ingested lithium is ≈95% (21, 22).

Analytic methods

Quercetin, kaempferol, and their conjugates in plasma or urine were simultaneously extracted and hydrolyzed to the aglycone by using 2 mol HCl/L in aqueous methanol (16) and determined by HPLC with fluorescence detection (23). The limit of detection was 2 μg/L for quercetin and 0.6 μg/L for kaempferol. Duplicate and control assays were run to assess the measurement error of the analytic method. CVs for duplicate assays were 4% for plasma and 6% for urinary quercetin and for the control samples were 10% for plasma quercetin (n = 18), 10% for urinary quercetin (n = 15), and 10% for plasma kaempferol (n = 3). Lithium was analyzed in a separate, undiluted, acidified urine sample by atomic-absorption spectrophotometry (24).

Statistical analysis

Data are reported as means ± SDs. To achieve normality we converted the amounts of quercetin analyzed in urine to log10 values. We determined within- and between-subject CVs as a measure of reproducibility for quercetin concentrations in plasma and urine after the 2 treatments with onions by using the SAS (SAS Institute Inc, Cary, NC) procedure VARCOMP. We calculated CIs for Pearson correlation coefficients by using Fisher’s z transformation.

RESULTS

Variability and reproducibility

Reproducibility of the 2 treatments with onions was similar for quercetin in both plasma and urine. Between the first and the
second onion treatment (with the same intake) the CV within subjects was 20% for plasma concentrations and 22% for urinary excretion. The CV between subjects was lower for plasma than for urine; it was 23% for concentrations in plasma and 36% for excretion in urine.

The correlation between the concentrations of quercetin in plasma and the amount excreted in urine, taken as the average for each individual after consumption of tea and onions, was 0.46. It was higher than that for kaempferol after tea, which was 0.19. However, when we dropped 1 outlying value, the correlation for kaempferol increased to 0.43. The individual excretion values for quercetin after consumption of tea and after onions correlated well. The Pearson correlation coefficient was 0.77 (Figure 1).

**Quercetin and kaempferol in plasma and urine**

The concentrations of quercetin in plasma after consumption of tea and after onions and of kaempferol after tea were increased in all subjects. The concentration of quercetin in plasma was on average 4 times higher after tea and 3 times higher after onions than at baseline (Table 1). The concentration of kaempferol in plasma after tea was on average > 6 times higher than the surrogate baseline concentration after onions (Table 2).

The amount of quercetin excreted in urine after consumption of tea was 8 times higher and after onions 4 times higher than that after the diet low in quercetin (Table 1). However, if both were expressed as a percentage of the amount consumed (Table 1 and Figure 2), the relative excretion of quercetin after onions was twice as high as that after tea. Differences in total urinary excretion of quercetin between treatment and baseline were on average 230 – 158 mg/d after tea and 124 – 85 mg/d after onions.

The amount of kaempferol excreted in urine after consumption of tea was 25 times higher than at baseline, and 7 times higher than after onions (Table 2). If expressed as a percentage of intake, the relative excretion of kaempferol after tea consumption was twice that of quercetin. Differences in excretion of kaempferol between treatment and baseline were 586 ± 197 µg/d after tea and 74 ± 68 µg/d after onions.

**Compliance with diets and completeness of urine collection**

The subjects consumed all the tea or onions, except for 1 subject who did not drink all her tea on the third day of the tea period. The average intake of quercetin plus kaempferol from the background diets was low, as determined by 24-h recalls. It was 2 ± 2 mg/d for the baseline period (n = 8) and 2 ± 2 mg/d for all treatment periods combined (n = 3 × 15). Lithium recoveries were 87 ± 13% (x ± SD; n = 8) in the baseline period, 93 ± 11% (n = 15) after tea consumption, and 97 ± 9% (n = 15) after the first and 96 ± 6% (n = 15) after the second treatment with onions.

**DISCUSSION**

**Biomarkers of intake**

The first objective of our study was to determine whether concentrations of flavonols in plasma and urine may be used as biomarkers of their intake. Our results suggest that plasma concentrations of quercetin and kaempferol and their excretion in urine can indeed be used as markers of their intake. A prerequisite of a biomarker is that differences in consumption can be discriminated (17). In our study, the consumption of quercetin and kaempferol from tea and onions increased their plasma concentrations and urinary excretion 3–25-fold. A low quercetin intake—as from the background diet—resulted in low amounts of quercetin, and an average intake of quercetin—as after onions, which provided 13 mg or 80% of the average daily Dutch consumption of quercetin—elevated plasma and urine concentrations of quercetin clearly in all subjects compared with baseline. However, a low intake of kaempferol, as after onions, which do not

**FIGURE 1.** Correlation between the excretion of quercetin in 24-h urine samples after consumption of tea and after consumption of onions. The subjects consumed 1600 mL tea, providing 49 mg quercetin/d, and 129 g onions, providing 13 mg quercetin/d, each for 3 d. Quercetin excretion values after onions are the average of 2 treatments. n = 15 subjects.
TABLE 1
Effect of the consumption of tea or onions on concentrations of quercetin in plasma and 24-h urine samples

<table>
<thead>
<tr>
<th>Supplement type and amount of quercetin (mg/d)</th>
<th>Quercetin in plasma</th>
<th>Quercetin in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/L</td>
<td>µg/d</td>
</tr>
<tr>
<td>Baseline, 0</td>
<td>7 ± 4</td>
<td>30 ± 11</td>
</tr>
<tr>
<td>Tea, 49 ± 4</td>
<td>29 ± 8</td>
<td>258 ± 150</td>
</tr>
<tr>
<td>Onions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1, 13 ± 1</td>
<td>22 ± 5</td>
<td>140 ± 66</td>
</tr>
<tr>
<td>Period 2, 13 ± 1</td>
<td>22 ± 7</td>
<td>131 ± 72</td>
</tr>
<tr>
<td>Average, 13 ± 1</td>
<td>22 ± 5</td>
<td>135 ± 66</td>
</tr>
</tbody>
</table>

1 \( \bar{x} \pm SD; n = 15. 
2 Expressed as the aglycone. 
3 \( n = 8. 
4 The onion supplement was repeated once to assess variability.

contain kaempferol, did not result in low excretion of kaempferol (Table 2). This may be explained by consumption of kaempferol-rich vegetables in the background diet by some subjects. A high intake of quercetin and kaempferol—as after tea in our study—resulted in the highest plasma and urine concentrations of both flavonols. Thus, our study shows that amounts of quercetin and kaempferol in plasma and urine reflected amounts of intake.

Another requirement for a biomarker is a certain degree of precision with which intake can be assessed. To evaluate this, the CV of a single estimate in a single subject must be determined. The CV for quercetin in plasma or urine includes laboratory error, random biological fluctuations within subjects, and variations between subjects due to differences in absorption and metabolism. The correlation of 0.77 between urinary excretion of quercetin after consumption of onions and that after tea showed that there are indeed consistent differences in flavonoid metabolism between subjects (Figure 1); subjects with low or high excretion after consumption of onions also showed low or high excretion after tea.

For plasma, the CV between 2 measurements of the same intake of quercetin from onions was 20% within subjects. The CV between subjects was 23%. Thus, the total observed CV of quercetin in plasma sampled once at the same intake can be estimated as \( \sqrt{0.2^2 + 0.23^2} = 30\% \). A similar calculation yields a total CV of 42% for the measurement of quercetin in urine. Whether this measurement error has to be considered as large or small can only be evaluated in relation to the actual variation in intake found between free-living subjects. From various studies (5, 6, 14, 25) it can be estimated that the CV for true differences in intake between subjects in the population is \( \approx 60\% \). This suggests that the random error in our proposed biomarkers is modest relative to the expected variations due to true variation in intake. We will illustrate the degree of precision of quercetin in plasma and urine as a biomarker of quercetin intake for 2 hypothetical examples with practical applications.

The first example addresses assessment of the compliance of a subject in an intervention study with a diet that provides 50 mg quercetin/d. The interval in which plasma quercetin of 1 of the subjects is predicted to lie with a probability of 95% is \( \mu \pm 1.96 \times \sigma \), where \( \mu \) denotes the expected mean concentration and \( \sigma \) the SD of the measurement (26). After an intake of 13 mg quercetin/d, the average concentration of quercetin in plasma in our subjects was 22 µg/L, with a CV of 30%. If we assume (de Vries et al, unpublished observations, 1997) that the concentrations of quercetin in plasma and urine increase linearly with the dose, then the expected concentration in plasma of a subject with an intake of 50 mg/d is 22 \( \times \) 50/13 = 85 µg/L. If the CV is the same at various concentrations, then the SD will be 30% of 85 µg/L, or 25.5 µg/L, and the 95% CI will range from 85 – 1.96 \( \times \) 25.5 = 35 µg/L to 85 + 1.96 \( \times \) 25.5 = 135 µg/L. Thus, there is reason to doubt dietary compliance of the subject if a single plasma quercetin concentration is < 35 µg/L. Better precision can be achieved by taking more plasma samples: for a mean of 2 measurements the lower boundary point for compliance would be 50 µg/L.

**Bioavailability**

The second example addresses the correlation coefficient between quercetin intake and a continuous variable, eg, the oxidizability of LDL. For this we estimate the attenuation factor of the correlation coefficient caused by measurement error. The attenuation factor is the ratio of the observed correlation to the “true” correlation. The factor is calculated as \( \sqrt{1/(1+(30\%/(1 \times \sigma^2))} \), in which \( \sigma^2 \) denotes the variance of the measurement, \( \sigma^2 \), the true variance of intake between subjects, and \( n \) the number of measurements (26, 27). From our data we estimate that the attenuation factor for a single measurement of quercetin in plasma or urine is \( \sqrt{1/(1+30\%/(1 \times 60\%)} = 0.82 \) for plasma and \( \sqrt{1/(1+43\%/(1 \times 60\%)} = 0.76 \) for urine. This implies that when the true correlation between quercetin intake and, for example, lagtime of LDL oxidation is 0.5, the observed correlation will be 0.4. The attenuation factors we found for the quercetin markers are similar to those for intake measurements of energy and various nutrients by the crosscheck dietary history method (28).

The second objective of our study was to determine whether the bioavailability of flavonols from tea is similar to that from onions. Our results show that quercetin and kaempferol from tea are absorbed, but that the bioavailability of quercetin from tea is less than that from onions. Plasma concentrations of quercetin were 30% and urinary excretions 100% higher after consumption of tea than after onions, but the intake from tea was about 4 times higher than from onions. When adjusted for intake—as shown by the urinary excretion relative to the amount consumed (Table 1)—the absorption from tea was about half that from onions. The reason could be that the absorption as a percentage of intake of a higher dose is lower than that from a lower dose, but it is more likely that the difference in type of quercetin conjugate present in the two foods plays a role. The absorption of quercetin rutinoside, a major quercetin compound in tea, is probably less than that of quercetin glucoside, the major compound in onions.
This agrees with the differences in absorption we found between these two compounds in previous studies (15). In ileostomy patients, quercetin in 13-h urine collections was 0.3% of intake after consumption of onions and 0.1% after quercetin rutinoside (15). The excretion in the present study was higher, which could be explained by enhanced absorption due to a lower dose or to bacterial activity in the colon that is not present in ileostomy patients. In a previous study in healthy volunteers (16), the relative excretion was 1.4% after an onion-rich breakfast and 0.4% after a breakfast rich in quercetin rutinoside. Thus, in all studies the relative excretion of quercetin in urine after consumption of onions was higher than after quercetin rutinoside or after tea, which confirms that quercetin from onions is better absorbed.

Incomplete collection of urine cannot explain the lower absorption of quercetin observed after consumption of tea. The average recoveries of lithium agreed with the expected value of 95%. Deviations in sampling by some subjects probably decreased the excretion of quercetin after tea, but after correction for incomplete collection the excretion of quercetin relative to intake after tea became 0.6% instead of 0.5%. Also, exclusion of the data from 1 subject who became ill during the experiment did not affect the results.

The excretion of unchanged kaempferol in urine was 2.5% of the amount consumed and that of quercetin only 1%. Therefore, kaempferol was absorbed better or metabolized to a lesser extent than was quercetin.

Utility of the biomarkers

Epidemiologic studies that investigate the relation between intake and disease require measurements of long-term intake (17). Flavonol markers probably do not reflect long-term flavonol intake. Quercetin and kaempferol accumulate in plasma after repeated intake of onions, apples, and tea (16), but the elimination half-life of about 20 h (16) indicates that a steady state concentration in plasma is reached after 4 d and that plasma concentrations would reflect intake of only the previous 3 d. Thus, repeated measurements in time may be needed to obtain an estimate that represents long-term intake. That would also reduce the error associated with variations in the interval

### TABLE 2
Effect of the consumption of tea or onions on concentrations of kaempferol in plasma and 24-h urine samples

<table>
<thead>
<tr>
<th>Supplement type and amount of kaempferol (mg/d)</th>
<th>Kaempferol in plasma</th>
<th>Kaempferol in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/L</td>
<td>µg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% of intake</td>
</tr>
<tr>
<td>Baseline, 0</td>
<td>ND</td>
<td>26 ± 7*</td>
</tr>
<tr>
<td>Tea, 27 ± 2</td>
<td>15 ± 5</td>
<td>668 ± 360</td>
</tr>
<tr>
<td>Onions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1, &lt;2</td>
<td>3 ± 4</td>
<td>123 ± 157</td>
</tr>
<tr>
<td>Period 2, &lt;2</td>
<td>1 ± 1</td>
<td>63 ± 27</td>
</tr>
<tr>
<td>Average, &lt;2</td>
<td>2 ± 2</td>
<td>93 ± 75</td>
</tr>
</tbody>
</table>

1* x ± SD; n = 15. ND, not determined.
2 n = 8.
3 The onion supplement was repeated once to assess variability.

### FIGURE 2
The amount of quercetin excreted in 24-h urine samples after consumption of tea and after consumption of onions as a percentage of the amount consumed per subject, corrected for baseline excretion. Data are presented for the 8 subjects whose baseline excretion values were known because they participated in the follow-up study. Quercetin excretion values after onions are the averages of 2 treatments.
between blood sampling and the most recent intake of flavonol-rich foods. Because of these short-term variations we may have missed the peak quercetin concentrations in plasma for onions as well for tea (16).

Whether the use of a biomarker is feasible also depends on the cost of measuring it and its stability in stored samples. A method for chemical determination of quercetin and kaempferol in plasma was developed by Hollman et al (23) and can now be conducted economically. The maximum decrease in quercetin content of stored plasma samples after 1 y was ≈10% (PCH Hollman, unpublished data, 1997). We expect the same for plasma kaempferol. Therefore, a long interval between sample collection and chemical analysis would not be a problem (17).

Plasma concentrations and urinary excretion of quercetin and kaempferol may also be regarded as markers of flavonol status in the body, rather than of flavonol intake. Therefore, these concentrations—when assessing the relation between flavonols and disease—may be more directly related to the outcome of disease than are intake data. In this way, differences in absorption between subjects and bioavailability between dietary sources of flavonols are taken into account.

Conclusion

We conclude that differences in short-term flavonol intake can be discriminated by the concentrations of quercetin and kaempferol in plasma and urine. The reproducibility of quercetin concentrations in plasma and urine suggest that these concentrations can be used as biomarkers to determine quercetin intake in epidemiologic studies. We also conclude that flavonols from tea are absorbed but that the bioavailability of quercetin from tea is only half of that from onions.

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REFERENCES