Flavonoids in Human Urine as Biomarkers for Intake of Fruits and Vegetables

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Abstract

Flavonoids are polyphenolic compounds ubiquitously found in human diets. We have studied the association between urinary excretion of flavonoids and the intake of fruits and vegetables to evaluate the usefulness of flavonoids as a biomarker for fruit and vegetable intake. Levels of 12 dietary relevant flavonoids were determined by LC-MS in urine samples collected prior to an intervention study, when the subjects were on their habitual diet (n = 94), and after they had participated in an intervention study with diets either high or low in fruits, berries, and vegetables (n = 77). Both flavonoid glycosides and aglycones were included in the assay, but only the flavonoid aglycones were detectable. Thus, the flavonols quercetin, kaempferol, isorhamnetin, and tamarixetin, the dihydrochalcone phloretin, and the flavanones naringenin and hesperetin were quantified in the enzymatically hydrolyzed urine samples.

The habitual intake of fruits and vegetables, determined by 3-day dietary records before the intervention study, correlated significantly with the total excretion of urinary flavonoids, with a coefficient of correlation of 0.35, P < 0.005 (n = 94). In addition, highly significant differences in the urinary excretion of all flavonoids were observed in the human intervention study between subjects on diets high or low in fruits, berries, and vegetables. Also, at the individual level a significant positive correlation between changes in fruit and vegetable intake and changes in urinary flavonoid excretion was observed. We conclude that urinary flavonoids may be useful as a new biomarker for fruit, berry, and vegetable intakes and may prove useful when the possible health protective effects of flavonoids are studied.

Introduction

A high intake of vegetables and fruits is associated with a reduced risk of many cancers and of coronary heart disease (1–4). It is still a matter of dispute whether there are certain vegetable or fruit groups or some specific compounds in vegetables and fruits that are responsible for the protection. Some studies even suggest that fruit and vegetable intake may be merely a marker of an otherwise healthful behavior (5). Furthermore, there are many uncertainties associated with the dietary intake assessment methods currently used in epidemiological studies. To be able to get better insight into the health effects of vegetables and fruits, reliable biological markers for the intake of vegetables or fruits are needed.

Apart from vitamins, minerals, and dietary fibers, vegetables and fruits contain several types of potentially bioactive compounds, e.g., the flavonoids, which may have protective effects as such. Flavonoids are a group of polyphenolic compounds with proven antioxidative properties in vitro. They are ubiquitously found in commonly consumed fruits, vegetables, and beverages such as wine and tea. A high intake of flavonoids or flavonoid-rich foods has been inversely associated with subsequent heart disease in some prospective studies (6–8). The widespread occurrence of flavonoids in the human diet and their ability to enter the systemic circulation make the determination of a representative group of flavonoids in biological samples interesting, not only for their potential health-protective effects but also as a very promising candidate to objectively assess the intake of fruits and vegetables. Plasma carotenoids have been used as markers for fruit and vegetable intake because of the ubiquitous distribution of these pigments in plants. However, plasma carotenoids have often shown only weak correlations with the intake of fruits and vegetables, probably because of individual variations in response to intake and other dietary factors influencing digestion and absorption (9). A more reliable biomarker for the intake of vegetables and fruits is thus warranted.

Research on flavonoids in relation to the human diet has mainly focused on the flavonol quercetin, primarily because of early methodologies enabling sensitive detection of this compound in biological fluids and in foods (10, 11). More recently, it has been found that other groups of flavonoids, such as the citrus flavanones, may be more important dietary constituents than the flavonols. The flavanones are major contributors to the total dietary intake of flavonoids in Denmark (12) and in Finland (13), for example, and may be absorbed to a much higher extent than flavonols, such as quercetin (14–16).

To evaluate the influence of flavonoids in our diet and their usefulness as a marker of certain vegetable or fruit groups, it is crucial to monitor the concentration of the major dietary...
flavonoids concurrently in human biological samples. Recently, a LC-MS assay has been developed (17), that simultaneously can quantify low levels of important dietary flavonoids in human urine samples with limited sample preparation. The chosen flavonoids belong to the groups of flavanones found in citrus fruits (naringin, naringenin, and hesperetin); flavonols, found in onions, apples, tea, cruciferous vegetables, and wine (quercetin-3-O-glucoside, quercetin-3-O-galactoside, rutin, quercetin, kaempferol, isorhamnetin and tamarixetin); and the dihydrochalcones, found in apples (phloridzin, and phloretin; Fig. 1). In the present study, the usefulness of total urinary flavonoids as a biomarker for the intake of fruits and vegetables was assessed by determining the concentration of these flavonoids in urine from subjects on their habitual diet and after a dietary intervention study with diets either high or low in FBV.

Materials and Methods

The urinary samples were obtained from a strictly controlled human dietary intervention study that focused on the effects of linoleic and oleic acids and vegetables, berries, and apple on several biochemical factors associated with the risk of coronary heart disease and cancer. The dietary intervention was carried out at the Division of Nutrition, University of Helsinki. The Ethics Committee of the Faculty of Agriculture and Forestry approved the study protocol.

Subjects. The volunteers were recruited among students and employees from the university campus, and their health status was checked with a questionnaire and screening tests (body weight and height, blood pressure, and urinary glucose and protein). Altogether 101 volunteers participated in the study, complete habitual diet and urinary flavonoid data were obtained during the pre-experimental period from 94 volunteers (71 females and 23 males) whose basic characteristics were: mean age, 26.6 years (median, 24 years; range, 19–52 years); and mean BMI, 22.7 kg/m² (median, 22.4 kg/m²; range, 18.3–34.1 kg/m²). During the experimental period, part of the volunteers served as control subjects and remained on their habitual diet, and the rest were randomized into four dietary intervention groups. Experimental diets were completed by 77 subjects. Of these subjects, 38 were on low FBV diets (28 females and 10 males); the mean age of these subjects was 25.5 years (median, 23 years; range, 19–52 years), and the mean BMI was 22.6 kg/m² (median, 22.4 kg/m²; range, 18.3–34.1 kg/m²). Thirty-nine subjects (29 females and 10 males), mean age of 24.8 years (median, 21 years; range, 19–50 years) and mean BMI of 22.7 kg/m² (median, 21.9 kg/m²; range, 17.9–33.7 kg/m²) completed high FBV diets. Four of the volunteers were smokers, and oral contraceptives were used by 46% of the female subjects. All subjects gave their informed consent after carefully reading the study protocol. The study foods were free to the subjects; otherwise, they received no payment.

Fig. 1. Chemical structures of all 12 dietary flavonoids used in the LC-MS assay and the internal standards 5,7,8-trihydroxyflavone and morin.

A. Flavanone

1. Naringin: R₁ = neohesperidose, R₂ = H, R₃ = OH

2. Naringenin: R₁ = H, R₂ = H, R₃ = OH

3. Hesperetin: R₁ = H, R₂ = OH, R₃ = OCH₃

B. Dihydrochalcone

4. Phloretin: R₄ = H

5. Phloridzin: R₄ = Glc

C. Flavanols

6. Rutin: R₁ = rutinoside, R₅ = H, R₆ = OH, R₇ = OH

7. Quercetin-3-O-Gal: R₅ = Gal, R₆ = H, R₇ = OH, R₇ = OH

8. Quercetin-3-O-Glc: R₅ = Glc, R₆ = H, R₇ = OH, R₇ = OH

9. Morin (IS): R₅ = H, R₆ = OH, R₇ = H, R₇ = OH

10. Quercetin: R₅ = H, R₆ = H, R₇ = OH, R₇ = OH

11. Kaempferol: R₅ = H, R₆ = H, R₇ = H, R₇ = OH

12. Isorhamnetin: R₅ = H, R₆ = H, R₇ = OCH₃, R₇ = OH

D. Flavone

13. Tamarixetin: R₁ = H, R₆ = H, R₇ = OH, R₇ = OCH₃

14. 5,7,8-trihydroxyflavone (IS)

Gal = galactose, Glc = glucose. IS = Internal standard.

The abbreviations used are: LC-MS, liquid chromatography-mass spectrometry; FBV, fruits, berries, and vegetables; BMI, body mass index; en%, 90% of the daily energy intake; HPLC, high-performance liquid chromatography; HAB, habitual diet during pre-experimental period; EXP, experimental diet during the 6-week intervention study.
Habitual Diet. The habitual diet of all subjects was evaluated with a 3-day food record using a picture booklet for the estimation of portion sizes (18), and intakes of different food groups were calculated from the Fineli database of Finnish foods. Data were examined for possible recording errors by checking for very high and very low food and nutrient intakes. The Fineli food groups were further pooled into the major groups of food items presented in Table 1.

Dietary Intervention. The intervention study was carried out in parallel so that each subject was on one experimental diet for 6 weeks. The experimental design is explained in more details elsewhere (19). The experimental foods provided 90 en% and were weighed for each participant according to individual energy needs to avoid weight changes. In addition to the study foods, the subjects had to choose from a limited list 10 en% as foods free of fat and cholesterol and low in antioxidants (sweets, sugar, cereal products, limited amount of alcoholic beverages (excluding red wine and berry wines), soft drinks, and a very limited selection of vegetables/fruits. The consumption of these foods was recorded in study diaries, as was habitual coffee and tea consumption. The subjects were advised to keep their lifestyle unchanged throughout the study, and the stability of body weight was monitored regularly. The experimental diets consisted of normal foods and were rich in linoleic acid (10 en%); diets P1 and P2) or oleic acid (12 en%; diets M1 and M2) and contained large (diets P2 and M2) or small (diets P1 and M1) amounts of FBV. The low FBV diets P1 and M1 contained no berries and included only small amounts of vegetables, mainly carrots, cabbage, and cucumber (total, 16.7 g/MJ/day), fruits (peeled apple, orange and banana; total, 5.4 g/MJ/day), and 29.4 g/MJ/day processed fruit and berry products (jam, marmalade, and apple juice). The high FBV diets P2 and M2 contained a mixture of nine different berries (strawberry, raspberry, bilberry, black currant, lingonberry, red currant, sea buckthorn berry, cloudberry, and crawberry; 20.3 g/MJ/day), 44 g/MJ/day of vegetables, including tomato, broccoli, onion, cabbage, kale, French beans, peas, and spinach, 16.6 g/MJ/day fresh unpeeled apple, and 25 g/MJ/day processed fruits and berries, mainly orange juice. All berries and apples as well as most vegetables were of Finnish origin and were included in the diet as little processing as possible. Energy provided from vegetables, berries, and apples in the high FBV diets were largely replaced by foods rich in sugar or starch (e.g., sugar, wheat bread, pasta, and rice) in the low FBV diets. Fiber intake was thus not balanced between the diets. The intakes of total fat (33 en%), saturated fat (11 en%), n-3 fatty acids (0.4 en%), protein (13 en%), and total carbohydrates (54 en%, including fiber) were calculated to be similar in all diets. The average intakes of different foods in the low (P1 and M1) and high (P2 and M2) FBV groups are shown in Table 1.

Collection of Urine Samples. Three consecutive 24-h urine samples were collected immediately after the habitual diet registration and during the last days of the dietary intervention period with urine-collecting aliquot cups (Daisho Co. Ltd, Osaka, Japan). With this equipment, 1/21 of the total urine volume was sampled, and the rest was discarded. Ascorbic acid (70 mg) was preweighed in each sample container (maximum volume, 100 ml). The subjects brought the samples daily to the Division of Nutrition. The volunteers were very carefully instructed how to use the urine collection cups, and the importance of complete samples was greatly emphasized. The volumes were measured, and to 7-ml aliquots of each sample were

### Table 1: Average intakes of different food groups during the habitual and experimental diets

<table>
<thead>
<tr>
<th>Food items</th>
<th>Habitual diet (n = 94) Mean ± SD (range) (g/day)</th>
<th>Experimental diets</th>
<th>Low FBV (n = 38) Mean ± SD (range) (g/day)</th>
<th>High FBV (n = 39) Mean ± SD (range) (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>197 ± 66 (57–367)</td>
<td>226 ± 42 (181–351)</td>
<td>184 ± 33 (137–289)</td>
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<tr>
<td>Oils, fat, and butter</td>
<td>35 ± 19 (8–96)</td>
<td>49 ± 9 (38–74)</td>
<td>46 ± 8 (34–72)</td>
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<tr>
<td>Dairy products</td>
<td>354 ± 230 (17–1262)</td>
<td>503 ± 85 (455–801)</td>
<td>485 ± 76 (439–777)</td>
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<tr>
<td>Meat, fish, and egg</td>
<td>145 ± 79 (6–458)</td>
<td>120 ± 22 (95–185)</td>
<td>115 ± 21 (83–180)</td>
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<tr>
<td>Sugar</td>
<td>14 ± 11 (0–48)</td>
<td>26 ± 5 (21–41)</td>
<td>20 ± 4 (16–31)</td>
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<tr>
<td>Coffee</td>
<td>147 ± 191 (0–740)</td>
<td>158 ± 261 (0–1132)</td>
<td>151 ± 179 (0–714)</td>
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<tr>
<td>Tea (Camellia sinensis and others)</td>
<td>156 ± 198 (0–900)</td>
<td>207 ± 145 (75–618)</td>
<td>185 ± 141 (75–654)</td>
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<tr>
<td>Alcoholic beverages</td>
<td>184 ± 316 (0–1884)</td>
<td>61 ± 58 (0–244)</td>
<td>101 ± 97 (0–513)</td>
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<tr>
<td>Potatoes (A)</td>
<td>98 ± 73 (0–352)</td>
<td>75 ± 14 (58–115)</td>
<td>59 ± 11 (43–92)</td>
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<tr>
<td>Roots (B)</td>
<td>34 ± 45 (0–293)</td>
<td>41 ± 7 (34–61)</td>
<td>108 ± 18 (80–159)</td>
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<tr>
<td>Other vegetables (C)</td>
<td>161 ± 83 (19–447)</td>
<td>118 ± 20 (97–174)</td>
<td>302 ± 50 (228–452)</td>
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<tr>
<td>Legumes (D)</td>
<td>11 ± 16 (0–67)</td>
<td>7 ± 1 (6–11)</td>
<td>31 ± 6 (23–48)</td>
<td></td>
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<tr>
<td>Fresh fruits (E)</td>
<td>137 ± 110 (0–663)</td>
<td>38 ± 6 (29–54)</td>
<td>163 ± 27 (120–228)</td>
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</tr>
<tr>
<td>Dried or canned fruits (F)</td>
<td></td>
<td>52 ± 8 (40–74)</td>
<td>14 ± 2 (11–21)</td>
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</tr>
<tr>
<td>Fruit juices (G)</td>
<td>108 ± 134 (0–550)</td>
<td>114 ± 23 (105–196)</td>
<td>154 ± 30 (143–286)</td>
<td></td>
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<tr>
<td>Fresh berries (H)</td>
<td>19 ± 44 (0–370)</td>
<td>0</td>
<td>206 ± 34 (154–306)</td>
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</tr>
<tr>
<td>Berry soup, jam, and juices (I)</td>
<td></td>
<td>97 ± 24 (79–190)</td>
<td>73 ± 19 (62–156)</td>
<td></td>
</tr>
<tr>
<td>Other juices (J)</td>
<td>96 ± 166 (0–867)</td>
<td>521 ± 264 (53–1673)</td>
<td>417 ± 79 (349–686)</td>
<td>913 ± 155 (725–1441)</td>
</tr>
<tr>
<td>Sum of FBV (sum of C and E to J)</td>
<td>521 ± 264 (53–1673)</td>
<td>417 ± 79 (349–686)</td>
<td>913 ± 155 (725–1441)</td>
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</tbody>
</table>

a Alcoholic beverages include beer, cider, wine, and spirits. Spirits consisted of <3% of the alcohol intake in both groups. The difference in alcohol intake between the groups is a result of higher beer intake in the high FBV group. No differences in wine intake were observed.
b In the low FBV diets was peeled apples, bananas, and apple juice instead of orange juice. In the high FBV diets, apples were unpeeled, bananas were excluded, and all fruit juice was orange juice.

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*National Public Health Institute, Finland http://www.ktl.fi/fineli.*
Flavonoids as Biomarkers for Fruit and Vegetable Intake

Differences between the pre-experimental levels in treatment groups (P1, P2, M1, and M2) were compared by Kruskall-Wallis one-way ANOVA. Because no differences in the baseline values were found between groups, the treatment effects were tested by comparing the experimental values between the treatment groups by Kruskall-Wallis one-way ANOVA and Mann-Whitney U test (data not shown). Because fatty acids did not affect urinary flavonoid excretion results (P1 versus M1 and P2 versus M2), the groups P1 and M1 were pooled as a low FBV group and groups P2 and M2 as a high FBV group to study the usefulness of urinary flavonoids as a marker of FBV intake. Mann-Whitney U tests were performed to compare the urinary flavonoid excretions in the high versus low FBV groups.

Although the FBV intake data on the habitual diet was less accurate than the very precise data available on FBV intake from the intervention study, attempts were made to investigate the effect of dietary changes on flavonoid excretion. Thus, the overall changes in FBV intake for each individual subject was expressed by the difference between the intake of FBV on the experimental diets (EXP) and the intake in the habitual period (HAB): \( \Delta \text{FBV} = \text{EXP} - \text{HAB} \). This was correlated with the corresponding individual changes in total flavonoid excretion (\( \Delta \text{Total flavonoid} = \text{EXP Total flavonoid} - \text{HAB Total flavonoid} \)).

Statview, Abacus Concepts Inc., version 4.53 (Berkeley, CA), and Systat statistical software package (Systat 5.2; SYSTAT, Inc., Evanston, IL) were used for the statistical analyses. Because of the several correlation analyses, \( P < 0.01 \) was considered significant in these comparisons. In other analyses, \( P < 0.05 \) was considered significant.

Results

The sensitivity and the selectivity of the LC-MS assay (17) proved more than sufficient to analyze urine samples from un-supplemented individuals. The assay allowed the simultaneous detection of both flavonoid glycosides and aglycones, but none of the included flavonoid glycosides were detectable in any of the analyzed urine samples. The flavonoids were determined after enzymatic hydrolysis of the urine, cleaving only sulfate and glucuronic acid conjugates of the flavonoids and not glycosides (20). Thus, the following flavonoid aglycones were determined in urine samples:

- flavonols: quercetin, kaemferol, isorhamnetin, and tamarixinetin;
- the dihydrochalcone phloretin;
- and the flavanones naringin, hesperetin, and morin;

The Spearman correlation analyses revealed some significant associations between individual and total flavonoids and relevant food groups. The significant correlations between fruit and vegetable intakes and urinary flavonoids are seen in Fig. 2. Removal of the outliers seen in the scattergrams in Fig. 2 only slightly reduced the correlation values and the significance levels, e.g., for total flavonoid versus FBV, \( r = 0.308, P = 0.003 \) with removal of the two outliers, and similarly, for hesperetin versus FBV, \( r = 0.334, P = 0.001 \). No correlation between the intake of berries and excretion of flavonoids was found, which probably is attributable to the very low intake of berries in the habitual diet. Significant associations were seen

\[ S. E. Nielsen, \text{ unpublished data.} \]
between excretion of kaempferol and intake of tea ($r = 0.485$, $P < 0.0001$), tea intake being also correlated with the excretion of quercetin ($r = 0.283$, $P < 0.005$). No significant associations were seen between flavonoid excretion and intakes of coffee, alcoholic beverages, roots, cereals, fats, dairy products, or meat and fish (data not shown). Impaired correlations for all of the individual flavonoids and for total flavonoids were seen when the intakes of legumes, roots, and potatoes were added to FBV. Potato intake showed significant negative correlation with kaempferol ($r = -0.269$, $P = 0.0096$) and nonsignificant negative correlations with the remaining flavonoids.

The association between total flavonoid excretion and the habitual intake of FBV was further investigated by dividing the data in quartiles according to FBV intake: $<326$ g/day ($n = 23$), $327–489$ g/day ($n = 24$), $490–639$ g/day ($n = 23$), and $>640$ g/day ($n = 24$). In Fig. 3, the mean excretion of flavonoids in these four groups is shown. Hesperetin, kaempferol, and total flavonoids tended to have dose dependent-like excretion, whereas naringenin, phloretin, and isorhamnetin showed significant differences only between quartiles 1 and 4. No significant differences were found, however, between the quartiles of quercetin and tamarixetin excretion.

Another way to study the relationship between fruit and vegetable intake and flavonoid excretion was to use the flavonoid excretion data from the strictly controlled diets. The dietary intervention was successful, and compliance to the experimental diets was good according to study diaries as well as analyses of plasma total fatty acids and plasma concentrations of carotenoids, quercetin, and vitamin C (19). Average energy intakes in the treatment groups did not differ, and the weight of the subjects remained constant during the experimental period.

The urinary flavonoid excretion in the experimental groups with low or high flavonoid diet periods is presented in Table 2. The data show that in the low FBV group, the excretion of all flavonoids decreased when compared with the habitual diet. In the high FBV group, the flavonoid excretion increased or tended to increase during the dietary intervention. At the end of the experimental period, flavonoid excretion levels were significantly higher in the high FBV group than in the low FBV group. No differences were seen in the control group during the experimental period (data not shown).

The scattergram in Fig. 4 shows a significant positive correlation between the changes in FBV intake ($\Delta$FBV) and flavonoid excretion ($\Delta$Total flavonoid), indicating that individual changes in dietary FBV intake was reflected in the urinary flavonoid excretion. Furthermore, changes in all of the individual flavonoids determined showed likewise significant positive Spearman correlations with $\Delta$FBV ($r = 0.339–0.501$, $P < 0.003$; data not shown).

### Discussion

The present study demonstrates, for the first time, that several different dietary flavonoids are measurable in urine from subjects on their habitual diet, and that the sum of flavonoids excreted in urine is associated with the intake of FBV. Urinary flavonoids may therefore be a valid biomarker for FBV intake. This is supported by the positive correlations between urinary flavonoid excretion and habitual FBV intake, the significant differences in flavonoid excretion between high and low FBV diets, and by the positive correlation between the deltas of FBV intake and flavonoid excretion during the dietary intervention.

The LC-MS analysis used in the present study allowed the simultaneous determination of seven different flavonoids originating from a wide variety of FBV in subjects with a realistic range of dietary intakes. Previous methodologies developed for flavonoid detection in biological fluids have been limited to the flavonols possessing fluorescence after postcolumn derivatization (11) or to a single flavonoid aglycone because of the high sensitivity and selectivity required of the method (20–22). Thus, investigations of several different flavonoids derived from normal unsupplemented human diets have until now not been possible.

In the present study, we used urine samples pooled from 3 consecutive days, reflecting the 3-day food registration. This was done to limit the number of samples and to increase the strength of each analyses as it reflects an average of 3 days instead of only a single day, thus reducing any reporting errors or uncompleted urine collections. Previous and ongoing studies suggest, however, that it is sufficient with urine collection from a single day, because the day-to-day variation at the individual level is limited (15). The amounts of excreted flavonoid aglycones (Table 2) are in good accordance with our previous reports on urinary excretion of flavonoids (15, 23). The excretion of both the citrus flavanones naringenin and hesperetin during the habitual diet and the experimental period was $\sim$10 times higher than the excretion of the flavonols quercetin, kaempferol, tamarixetin, and isorhamnetin. Both hesperetin and naringenin were also correlated with the FBV intake. This indicates that citrus fruits and juices markedly contribute to the

### Table 2 Flavonoid excretion in urine samples collected in the HAB or EXP period

<table>
<thead>
<tr>
<th>Flavonoid and study period</th>
<th>Study groups</th>
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<tbody>
<tr>
<td></td>
<td>Low FBV ($n = 38$)</td>
<td>High FBV ($n = 39$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
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<tr>
<td>Quercetin</td>
<td></td>
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<tr>
<td>HAB</td>
<td>25.1 ± 22.5</td>
<td>21.8 ± 17.4</td>
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<tr>
<td>EXP</td>
<td>12.8 ± 9.0$^b$</td>
<td>60.2 ± 33.1$^{b,c}$</td>
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<tr>
<td>Kaempferol</td>
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<tr>
<td>HAB</td>
<td>50.2 ± 32.1</td>
<td>52.3 ± 36.7</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>31.2 ± 19.8$^b$</td>
<td>91.6 ± 45.1$^{b,c}$</td>
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<tr>
<td>Isorhamnetin</td>
<td></td>
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<tr>
<td>HAB</td>
<td>11.2 ± 13.7</td>
<td>9.1 ± 6.3</td>
<td></td>
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<tr>
<td>EXP</td>
<td>4.2 ± 1.6$^b$</td>
<td>20.3 ± 17.2$^{b,c}$</td>
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<tr>
<td>Tamarixetin</td>
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<tr>
<td>HAB</td>
<td>11.4 ± 13.8</td>
<td>9.5 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>4.3 ± 2.0$^b$</td>
<td>20.6 ± 19.2$^{b,c}$</td>
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<tr>
<td>Naringenin</td>
<td></td>
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<tr>
<td>HAB</td>
<td>701.1 ± 658.5</td>
<td>607.8 ± 469.8</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>161.7 ± 191.9$^b$</td>
<td>695.4 ± 490.0$^b$</td>
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<tr>
<td>Hesperetin</td>
<td></td>
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<tr>
<td>HAB</td>
<td>762.6 ± 679.1</td>
<td>779.3 ± 36.7</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>122.2 ± 166.4$^b$</td>
<td>1133.7 ± 1131.4$^d$</td>
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<tr>
<td>Phloretin</td>
<td></td>
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<tr>
<td>HAB</td>
<td>76.0 ± 109.6</td>
<td>93.4 ± 136.1</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>5.3 ± 6.8$^b$</td>
<td>133.4 ± 101.0$^{b,c}$</td>
<td></td>
</tr>
<tr>
<td>Total flavonoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAB</td>
<td>1637.6 ± 1316.3</td>
<td>1573.3 ± 1277.0</td>
<td></td>
</tr>
<tr>
<td>EXP</td>
<td>341.6 ± 342.4$^{d}$</td>
<td>2155.2 ± 1629.9$^{d}$</td>
<td></td>
</tr>
</tbody>
</table>

* Determined in pooled 24-h urine samples collected over 3 consecutive days before and after a 6-week intervention period. Data are given as µg of excreted flavonoid in 24 h, for subjects during their habitual diet (pooled groups and study groups separately) or at the end of the 6-week experimental period.

* Significant difference within the study group between HAB and EXP values (Wilcoxon, $P < 0.01$).

* Significant difference between the values between the high and low FBV diets (Mann-Whitney U test, with $P < 0.001$).

* Significant difference within the study group between HAB and EXP values (Wilcoxon, $P < 0.05$).
total flavonoid intake in the Finnish diet, and that inclusion of the flavanones is important when assessing the total flavonoid intake. Although quercetin is widely distributed in plant species, it is generally present only in low concentrations, except for specific plant foods with very high quercetin content such as onions, cruciferous vegetables, and some berries (13, 24) that do not contribute considerably to the average fruit and vegetable intake in Finland (13). The weak correlation between quercetin and FBV ($r = 0.15$) indicates that quercetin alone was not a marker for total FBV intake in the present study.

Even although the present data suggest that the excretion of total flavonoids may be the best marker for the general FBV intake, individual flavonoids may serve as indicators of intake of specific food groups. This is illustrated by the significant correlations, e.g., between hesperetin and fruit juice intake, phloretin and fruit intake, or quercetin and the intake of vegetables. Kaempferol and quercetin correlated with tea intake, which is consistent with a quite high content of these two flavonols in tea (24).

The present study was not designed to study the bioavailability or kinetics of flavonoids. However, the fact that no flavonoid glycosides were detected in our samples seems to support previous studies reporting that the flavonoid glycosides are cleaved to the free aglycone and the sugar moiety before absorption by β-glucosidases in the small intestine or by microorganisms in the large intestine (25, 26). Endogenous metabolism resulting in configurational changes of the flavonoid aglycones has been reported to be of minor importance for the type of flavonoids determined in the present study (14, 23), and the major part of the excreted flavonoids probably result from flavonoids absorbed from the diet. Isoflavonoids and tamarixetin, however, may also result from endogenous metabolism of quercetin by catechol-O-methyltransferase (27).

When the habitual diet data were analyzed in FBV intake...
in France found correlation values of 0.36, \( P < 0.05 \), serum and absorption. A recent French study with response to intake and other dietary factors influencing digestion probably because of considerable individual variations in re-
have also been of various degrees of significance (9, 28), roots, where correlation coefficients of 0.483 and 0.315, re-
a biomarker of intake can be established. Flavonoid absorption and excretion rates include considerable interindividual varia-
tions to FBV intake, supporting that flavonoids might be a
combination of several flavonoids and carotenoids and maybe also be of some other plant-specific compound.

Determination of many different flavonoids belonging to different subclasses, as in the present study, may provide an important insight into the protective effects of dietary fla-
vonoids on cancer and coronary heart disease. Previous food analysis of the content of flavonols, primarily quercetin, in fruits and vegetables (10) and the application of these data on
large cohort studies have given conflicting results on the pro-	ective effects of quercetin on cancer and coronary heart disease (6–8, 34–36). However, very recent results indicate that the intake of quercetin (from onions and apples) in combination with other flavonoids, such as the citrus flavanones (from grapefruit), may reduce the risk of lung cancer (37), suggesting that several different flavonoids may contribute to the health-
protective effects of fruits and vegetables, supporting the im-
portance of looking at more than a single dietary flavonoid or
a single subclass of flavonoids. Further research will provide evidence, whether other flavonoids, such as quercetin, or combi-
inations of several flavonoids from different subclasses con-
tribute to the health-protective effects of a high dietary intake of
fruits and vegetables.

The present study has shown that urinary excretion of flavonoids is significantly correlated with the intake of fruits
vegetables and that urinary flavonoids may be a useful
biomarker for fruit and vegetable intake. The study has furth-
more shown the importance of inclusion of several different flavonoids when using flavonoids as a biomarker for FBV intake. The validity of urinary flavonoids as a biomarker of the intake of FBV should be further investigated in intervention and
cohort studies.

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Flavonoids in Human Urine as Biomarkers for Intake of Fruits and Vegetables

Salka E. Nielsen, Riitta Freese, Päivi Kleemola, et al.


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