Urinary Excretion of Flavonoids Reflects Even Small Changes in the Dietary Intake of Fruits and Vegetables

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Abstract

Background: Due to the random and systematic measurement errors associated with current dietary assessment instruments, there is a need to develop more objective methods of measuring the intake of foods of importance to human health. Objective: The purpose of this study was to test whether urinary excretion of flavonoids could be used to identify subjects who are meeting Norwegian recommendations for fruit and vegetable intake (5 servings per day) from individuals who are consuming the national average amount of fruits and vegetables (2 servings per day). Design: Twenty-four-hour urine samples were collected in a strict crossover controlled feeding study. Forty healthy subjects (19–34 years) were included in the study. After a 1-week run-in period, one group was given a controlled diet that included 2 servings (300 g) of fruits and vegetables daily for 14 days, while the other group was given a diet containing 5 servings (750 g) per day. Following a 2-week washout and a 1 week run-in period, the regimens were switched between the groups. Results: An increased intake of mixed fruits and vegetables from 2 to 5 servings per day significantly enhanced urinary excretion of eriodictyol, naringenin, hesperetin, quercetin, kaempferol, isorhamnetin, and tamarixetin. The citrus flavonoids naringenin and hesperetin showed a steep dose-response relationship to dietary intake of fruits and vegetables, whereas the association to eriodictyol, quercetin, kaempferol, isorhamnetin, and tamarixetin was more moderate. Conclusion: The present study indicates that urinary excretion of dietary flavonoids may be used to assess changes of mixed fruit and vegetable intake corresponding to an increase from the present national intake in Norway to the recommended amount of 5 servings of fruits and vegetables daily. (Cancer Epidemiol Biomarkers Prev 2004;13(5):843–9)

Introduction

High intake of fruits and vegetables has been shown to protect against development of many non-communicable diseases like several types of cancers and coronary heart disease (1–4). Accurate estimation of fruit and vegetable intake is critical to further study the association between intake and development of chronic disease. Food diaries, food frequency questionnaires, and dietary recalls represent traditional methods for dietary assessment. All of these methods are associated with large random and systematic errors (5–9). Blood and urine biomarkers for intake of foods may offer a more objective, universal, and physiologically relevant method for measuring intake. However, a thorough validation of the suggested biomarkers represents a critical and often underrated step in the development and maturation of new biomarkers. Due to large cultural and geographic variation in eating patterns, the validation of biomarkers for food items will have to include several controlled studies testing a large variety of eating patterns.

Several substances found in fruits and vegetables may be potential biomarkers, and serum concentrations of carotenoids and vitamin C have received most attention (10–18). However, these biomarkers have several limitations; the absorption of carotenoids is subject to high inter-individual variation (19, 20), and is affected by factors such as gender, body mass index, physical activity, and amount of fat in the diet (21–25). Moreover, plasma concentration of vitamin C has been shown to be affected by smoking and oxidative status (26). New candidate biomarkers are the flavonoids that are found ubiquitously in most fruits and vegetables. A recent parallel feeding study has demonstrated a correlation between total urinary excretion of flavonoids and the intake of fruits, berries, and vegetables (27). Beyond this study, the literature about flavonoids as biomarker of fruit and vegetable intake is scarce.

The aim of the present study was to investigate whether urinary excretion of flavonoids could be used as biomarkers for changes in intake of fruits and vegetables from the Norwegian average consumption (2 servings per day) to the recommended consumption
of fruits and vegetables (5 servings per day). A consider-
able inter-individual variation in flavonoid uptake and metabolism has been reported (28–30), and to reduce the importance of this variation, a crossover design was used.

**Materials and Methods**

The dietary intervention was carried out at the Institute for Nutrition Research at the School of Medicine, University of Oslo. The regional branch of The National Committee for Medical Research Ethics approved the study protocol.

Subjects were recruited among students from the Medical faculty, University of Oslo, Norway. Inclusion criteria were a body mass index <30 kg/m^2^, alcohol consumption <30 g/day, no use of vitamins or other food supplements the previous 2 months, age <35 years, no allergies, no prescribed medication (except contraceptives), no smoking, and no major fluctuations in body weight (<2 kg) during the last 2 years before baseline. Body mass index was calculated as weight (kilograms) divided by the height squared (meters). One person dropped out during the washout period between the two diet periods, and the total number of participants completing both dietary periods was 39. A crossover analysis is based on within-subject differences, the rationale being that subjects serve as their own control, and the analysis requires whole data series. Furthermore, one outlier was discarded from the flavonoid analysis because of extremely high baseline values. Data from 38 subjects were included in data evaluation. Baseline characteristics of the participants are shown in Table 1.

**Study Protocol.** We performed a randomized crossover study in the fall of 2001. Participants were stratified on the basis of gender and randomized into groups A and B. Group A was assigned to a diet containing 2 servings of fruits and vegetables (50 g) in the first diet period, lasting 14 days, while group B's diet in this period contained 5 servings of fruits and vegetables (750 g). Group A and B switched to 5 and 2 servings of fruits and vegetables, respectively, in the second 14-day diet period. The two diet periods were separated by a washout period of 14 days. During washout, the participants consumed a normal diet. Before each diet period, all participants had to go through a 7-day run-in period on a self-selected diet without fruits and vegetables. The run-in periods were included as an attempt to bring all participants to similar level with respect to baseline intake of fruits and vegetables. Thus, the overall study design was 1 week (run-in) + 2 weeks (diet period 1) + 2 weeks (washout) + 1 week (run-in) + 2 weeks (diet period 2).

**Diet Composition.** All subjects had diets with energy levels related to their energy needs. The relative content of energy from protein, fat, and carbohydrates was similar for all energy levels. The energy intake from breakfast, lunch, and snack meals was used to adjust the energy intake. Except from a small amount of jam, these meals were free of fruits and vegetables. Dinner and all the included fruits and vegetables were served and eaten under supervision of researchers at the Institute for Nutrition Research. Participants were free to choose where they wanted to consume their breakfast, lunch, and snack meals.

For breakfast and lunch, subjects were given bread or cereals along with small servings of jam and margarine. To escort the bread, different sorts of cheese and ham could be selected from a few predefined alternatives. Cake, buns, and chocolate (Saturday) were provided as snacks. Dinners were cycled between the following six different dishes: pasta carbonara, hash, baked fish, meat and potato, smoked salmon with sauce, and chicken curry with rice. All food items eaten in the two diet periods were supplied except for milk and spreads, which the participants were allowed to choose every day from

### Table 1. Baseline characteristics of participants in dietary intervention with fruits and vegetables

<table>
<thead>
<tr>
<th></th>
<th>Group A^a^</th>
<th>Group B^b^</th>
<th>Pooled baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Females</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Age (years), (\bar{x}) (range)</td>
<td>24 (20.0–34.0)</td>
<td>22.5 (19.0–30.0)</td>
<td>23.2 (19.0–34.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m^2^), (\bar{x}) (range)^c^</td>
<td>21.8 (19.3–26.3)</td>
<td>22.5 (18.3–27.8)</td>
<td>22.2 (18.3–27.8)</td>
</tr>
<tr>
<td>Basal metabolic rate (MJ), (\bar{x}) (range)^d^</td>
<td>6.5 (5.2–8.8)</td>
<td>6.8 (5.0–8.6)</td>
<td>6.7 (5.0–8.8)</td>
</tr>
</tbody>
</table>

Mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Group A^a^</th>
<th>Group B^b^</th>
<th>Pooled baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloretin (ng/ml)</td>
<td>47.9 (48.1)</td>
<td>33.0 (42.7)</td>
<td>40.5 (34.5)</td>
</tr>
<tr>
<td>Eriodictyol (ng/ml)</td>
<td>15.4 (26.2)</td>
<td>17.4 (24.0)</td>
<td>16.4 (20.8)</td>
</tr>
<tr>
<td>Naringenin (ng/ml)</td>
<td>32.0 (29.0)</td>
<td>22.3 (18.4)</td>
<td>27.1 (17.0)</td>
</tr>
<tr>
<td>Hesperetin (ng/ml)</td>
<td>9.2 (9.0)</td>
<td>9.7 (9.1)</td>
<td>9.4 (6.6)</td>
</tr>
<tr>
<td>Quercetin (ng/ml)</td>
<td>11.6 (10.9)</td>
<td>11.2 (11.0)</td>
<td>11.4 (7.7)</td>
</tr>
<tr>
<td>Kaempferol (ng/ml)</td>
<td>7.7 (9.3)</td>
<td>8.8 (12.0)</td>
<td>8.3 (7.5)</td>
</tr>
<tr>
<td>Isorhamnetin (ng/ml)</td>
<td>4.9 (1.0)</td>
<td>4.9 (1.7)</td>
<td>4.9 (1.0)</td>
</tr>
<tr>
<td>Tamariexetin (ng/ml)</td>
<td>5.0 (1.3)</td>
<td>4.9 (2.4)</td>
<td>5.0 (1.3)</td>
</tr>
</tbody>
</table>

Note: Flavonoids were measured in 24-h urine samples.

^a^Group A was given 2 servings of fruits and vegetables in the first period and 5 servings in the second period.

^b^Group B was given 5 servings in the first period and 2 servings in the second period.

^c^BMI was calculated as weight (kilograms) divided by the height squared (meters).

^d^BMR was calculated using WHO standard formulas (38).
a few alternatives. No additional foods or beverages, except for water, coffee, and tea, were allowed. Tea bags (one brand) were given to the participants, and they had to record the number of tea bags used and the amount of tea consumed during the diet periods. On weekends, all foods were supplied frozen in insulated plastic thermobags, except from a plate of milk chocolate which was handed out along with the weekend food packages.

The types of fruits and vegetables included are listed in Table 2. They were chosen to match a typical Norwegian diet (31, 32). All fruits and vegetables were from the same batch, and all fruit and vegetable servings were pre-prepared before the intervention and frozen at −20°C. The vegetables were lightly heated before serving. Nutrient losses during the freezing period were not measured. The relative amounts of different fruits and vegetables were the same in both the high and low fruit and vegetable diet period, and the fruit and vegetable combination served daily during a diet period was the same. Only the non-fruit and vegetable component of the dinner were cycled between six alternatives. All participants had the same main course for dinner on the same day. At the end of each diet period, all subjects had to report in a written diary if they had eaten all the supplied foods, what they had not eaten, and if they had eaten additional food not allowed according to the protocol. The written diaries also contained information about ad libitum consumption of tea and coffee during the diet periods.

Energy requirements were calculated on the basis of basal metabolic rate and patterns of physical activity obtained from a personal interview (33). Basal metabolic rate was calculated using standard formulas (34). During the diet periods, all subjects were weighed 3 times weekly. Dietary intake of total energy from fat, protein, and carbohydrates in the diet were calculated using a weekly. Dietary intake of total energy from fat, protein, carbohydrates in the diet were calculated using a newly developed software tool (KBS, version 3.1, 2002) and if they had eaten additional food not allowed according to the protocol. The written diaries also contained information about ad libitum consumption of tea and coffee during the diet periods.

Energy requirements were calculated on the basis of basal metabolic rate and patterns of physical activity obtained from a personal interview (33). Basal metabolic rate was calculated using standard formulas (34). During the diet periods, all subjects were weighed 3 times weekly. Dietary intake of total energy from fat, protein, and carbohydrates in the diet were calculated using a newly developed software tool (KBS, version 3.1, 2002) linked to the national food composition table. All unconsumed food items were subtracted and data from the participant’s actual consumption were used for calculation of total energy intake.

Collection of Urine Samples. Urine samples were collected on the day before the start and on the last day of each of the two diet periods. All subjects collected a total of four 24-h urine samples. Aliquots of 10 ml (10%) aqueous ascorbic acid and 50 ml (1 ml) HCl were pre-weighted in each sample container (2.5 l). In addition to the main sample container, all subjects were given a smaller 500 ml container with 2 ml (10%) aqueous ascorbic and 10 ml (1 ml) HCl that was easier to carry around. Total urine volumes were measured on delivery of the urine samples, and six 1-ml samples were stored at −70°C until analysis.

Reagents and Standards. Acetonitrile and methanol were of HPLC grade and obtained from Rathburne Ltd. (Walkerburn, United Kingdom). The flavonoid standards kaempferol, isorhamnetin, tamarixetin, genistein, and daidzein were all obtained from Apin Chemicals Ltd. (Oxon, United Kingdom). Phloretin and hesperetin were purchased from Sigma Chemicals Co. (St. Louis, MO) and quercetin and naringenin were from Aldrich (Steinheim, Germany). The isotopic-labelled internal standards, 3 × 13C labelled genistein and 3 × 13C labelled daidzein, were obtained from School of Chemistry, University of St. Andrews, United Kingdom. All standards were HPLC grade. A stock solution of 100 µg/ml of a mixture of all the flavonoid standards was prepared in DMSO. Stock solutions of the internal standards genistein, 3 × 13C labelled genistein, daidzein, and 3 × 13C labelled daidzein were prepared in DMSO, at concentrations of 20 µg/ml. All stock solutions were stored at −20°C and were stable for at least 3 months. The enzymes used for enzymatic hydrolysis of the urine samples were β-glucuronidase (Escherichia coli), >200 standard units/ml) obtained from Boehringer Mannheim (Mannheim, Germany) and arylsulfatase (Aerobacter aerogenes, 16.8 standard units/ml) from Sigma (St. Louis, MO). All other chemicals used were of HPLC or reagent grade.

Determination of Flavonoids. The urinary concentrations of eight dietary flavonoids were determined by HPLC-mass spectrometry (MS) using atmospheric pressure chemical ionization (APCI). The flavanones: eriodictyol, naringenin, and hesperetin; the flavonols: quercetin, kaempferol, isorhamnetin, and tamarixetin; and the dihydrochalcone phloretin were all determined simultaneously in each urine sample. The method is essentially described elsewhere (28), except for the inclusion of the determination of eriodictyol and the use of different internal standards in the present study. Eriodictyol was eluted with a retention time of 15.2 min and was detected at m/z 287 [M−1]−, and the fragment ion at m/z 151 was used as qualifier ion. In brief, 2-ml aliquots of the 24-h urine samples from each individual were added to 25 µl of a mixture of 500 ng of genistein and 500 ng 3 × 13C labelled genistein dissolved in DMSO as internal standards and enzymatically hydrolyzed as described elsewhere (28) Genistein and 3 × 13C labelled genistein was eluted at 22.6 min and was determined as [M−1]− at m/z 269 and 272, respectively. After hydrolysis, 2 ml ice-cold methanol were added to each sample to stop the reaction, and the samples were evaporated to dryness under vacuum. The hydrolyzed samples were redissolved in 10% aqueous methanol, 1% formic acid, and added 25 µl of a mixture of 500 ng of daidzein and 500 ng 3 × 13C labelled daidzein dissolved in DMSO.

### Table 2. Fruits and vegetables included in the experimental diet

<table>
<thead>
<tr>
<th>Foods</th>
<th>Low FV Diet (g/day)</th>
<th>High FV Diet (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspberry</td>
<td>6.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Blueberry</td>
<td>6.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Red pepper (cooked)</td>
<td>8.9</td>
<td>22.5</td>
</tr>
<tr>
<td>Pear</td>
<td>10.8</td>
<td>27.0</td>
</tr>
<tr>
<td>Canned tomatoes (cooked)</td>
<td>11.0</td>
<td>27.7</td>
</tr>
<tr>
<td>Jam (strawberry)</td>
<td>12.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Apple</td>
<td>19.4</td>
<td>48.6</td>
</tr>
<tr>
<td>Orange</td>
<td>20.5</td>
<td>51.3</td>
</tr>
<tr>
<td>Onion (cooked)</td>
<td>20.7</td>
<td>51.9</td>
</tr>
<tr>
<td>Tomato (cooked)</td>
<td>26.9</td>
<td>67.4</td>
</tr>
<tr>
<td>Broccoli (cooked)</td>
<td>27.5</td>
<td>80.0</td>
</tr>
<tr>
<td>Cauliflower (cooked)</td>
<td>27.5</td>
<td>47.0</td>
</tr>
<tr>
<td>Orange juice</td>
<td>46.4</td>
<td>116.1</td>
</tr>
<tr>
<td>Carrot (cooked)</td>
<td>56.0</td>
<td>140.0</td>
</tr>
<tr>
<td>Total fruits and vegetables</td>
<td>300.0</td>
<td>750.0</td>
</tr>
</tbody>
</table>
as additional internal standards, assessing the performance of the mass spectrometer, giving a final volume of 250 μl. Daidzein and the isotopic-labelled daidzein were detected at a retention time of 17.9 min and as the molecular ion [M−1] at m/z 269 and 272, respectively. The sample was then centrifuged at 10,000 × g for 5 min at 4°C and the entire amount of the supernatant was injected onto the HPLC-APCI-MS system. Before, and after each series of analysis, the performance of the entire LC-MS assay was controlled by injections of aliquots containing all employed flavonoid standards, including the internal standards as previously described (28). In addition, blank urine samples added to 250 ng of each of the analytes and the four internal standards (500 ng/sample) were included in the beginning, in the middle, and in the end of each series of 25 urine samples to evaluate performance of each run. The coefficient of variation % (CV%) of the repeatability of the assay was below 12% (n = 20). The laboratory personnel were blinded for all treatments.

**Statistical Analysis.** Data were ln transformed to obtain normality in the crossover analysis. The limit of quantification for all flavonoids determined (LOQ) in the LC-MS analysis was 5 ng/ml urine.

The analyses of a two-treatment crossover study can be presented as three two-sample t tests (35, 36). Before comparing the treatments, the possibility of a period effect and a treatment interaction effect (carry-over effect) were tested. A potential period effect was tested by a two-sample t test to compare differences between the diet-periods in the two groups of participants. Possible treatment-period interaction was investigated by noticing that in the absence of an interaction, a subject’s average response to the two diets should be similar regardless of the order in which the diets were given. The test for interaction is thus a two-sample t test comparing average for group A with average for group B. Because the two crossover groups were not the same size, the effect of the experimental diet was tested by performing a two-sample t test to compare the average differences between the two treatments. Mann-Whitney U test was used to test for possible differences between groups in the non-transformed baseline values. Most commentators would agree that the statistical procedure employed adjusts the treatment effect adequately for any difference due to periods and also eliminates the effect of individual subjects from the estimate of the SE of the treatment (37). The significance level was set to 5%, and all analyses were performed using SPSS 11.

**Results**

There were no significant differences between the baseline characteristics of the subjects allocated to group A or B, as seen in Table 1. The content of major nutrients and the energy intake was equal in the two diets providing either 2 or 5 servings of fruits and vegetables (Table 3). Compliance was good as judged from the written records and personal feedback. All experimental fruits and vegetables were consumed, except from one portion of 125 g of fruit salad forgotten by one subject in the first diet period. Two subjects consumed a mouthful of communion wine twice in each diet period. Except for these instances, all subjects were completely compliant to all dietary restriction and to the dietary treatments. All subjects had constant body weight during the study (<1.5 kg fluctuation).

Tea and coffee consumption did not differ in groups A and B during the diet periods, and was not related to the urinary excretion of flavonoids.

Concentration of the diet containing the recommended amount of 5 servings of fruits and vegetables per day gave significantly higher urinary excretion of the flavanoids eriodictyol, naringenin, hesperetin and the flavonols quercetin, kaempferol, isorhamnetin, and tamarixetin compared to the diet containing 2 servings of fruits and vegetables per day. Period effects were observed for tamarixetin and isorhamnetin and isorhamnetin was afflicted with a period-treatment interaction (Table 4). The statistical method automatically adjusts for period effects.

Figure 1 shows the absolute excretion in 24 h urine of all flavonoids measured at three periods; at baseline after the first run-in period where no fruit and vegetable were consumed, after the diet period with 2 servings of fruit and vegetable per day, and after the diet period with 5 servings per day. All flavonoids with a significant treatment effect in the crossover analyses demonstrated a dose-dependent increase in urinary excretion from baseline to the highest serving size (Fig. 1). The dose-response relationship was strongest for quercetin, isorhamnetin, naringenin, hesperetin, and total flavonoids. Moreover, the urinary concentrations of flavonols were lower than the flavanones. Naringenin and hesperetin responded more than any other flavonoid to the dietary treatment. The increase in flavonoid excretion compared to baseline was only significant after the highest fruit and vegetable dose (5 servings) for kaempferol, tamarixetin, and eriodictyol. Phloretin did not respond at all to the intervention.

**Discussion**

The main finding in this controlled crossover study is that urinary excretion of several flavonoids may be used to assess changes in intake of mixed fruits and vegetables corresponding to an increase from 2 servings to the recommended amount of 5 servings of mixed fruits and vegetables per day. For most of the flavonoids measured, there was also a significant increase from baseline to 2 servings (Fig. 1). To our knowledge, this is the first time the flavanone eriodictyol has been quantified in human urine.

**Table 3. Dietary composition during intervention**

<table>
<thead>
<tr>
<th></th>
<th>2 Servings/day (n = 38)</th>
<th>5 Servings/day (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Means (SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, % of energy</td>
<td>31.9 (1.7)</td>
<td>32.3 (1.7)</td>
</tr>
<tr>
<td>Protein, % of energy</td>
<td>18.0 (0.9)</td>
<td>17.8 (1.0)</td>
</tr>
<tr>
<td>Carbohydrate, % of energy</td>
<td>50.1 (2.4)</td>
<td>50.0 (2.3)</td>
</tr>
<tr>
<td>Energy intake, MJ</td>
<td>13.1 (3.1)</td>
<td>13.2 (3.1)</td>
</tr>
<tr>
<td>Fiber, g/day</td>
<td>35.4 (11.4)</td>
<td>43.7 (9.8)</td>
</tr>
<tr>
<td>Tea, ml/day</td>
<td>219 (197)</td>
<td>177 (135)</td>
</tr>
</tbody>
</table>

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There was a significant effect of number of servings on the urinary excretion of the flavanones naringenin, hesperetin, and eriodictyol (Table 4). The flavanones naringenin, hesperetin, and eriodictyol are abundant in citrus fruits and may thus be referred to as citrus flavonoids (27, 38, 39). The diet including 2 servings of fruits and vegetables contained 67 g of orange and orange juice per day, whereas the diet with 5 servings of fruits and vegetables contained 167 g orange and orange juice per day. Flavanone half-lives are short. In an experiment performed by Erlund et al. (29), 63% of total urinary excretion of naringenin and 65% of hesperetin were excreted in the 4–8 h fraction.

We also observed an effect of serving size on the flavonols quercetin, kaempferol, tamarixetin, and isorhamnetin (Table 4). The flavonols are found in onions, apples, tea, cruciferous vegetables, and wine (40). In an attempt to calculate the intake of quercetin in the Netherlands (40), tea turned out to be the major source (48% of total) followed by onions (29%) and apples (7%). However, no significant relationship between average intake of tea and urinary excretion of either quercetin or kaempferol was observed in this study. This is in contrast to what have been reported by others (27, 40, 41). The period effect and the generally low amount of excretion observed for tamarixetin and isorhamnetin, together with the period treatment interaction observed for isorhamnetin, indicates that tamarixetin and isorhamnetin may be less stable biomarkers for mixed fruit and vegetable intake than for example quercetin. This is probably due to the fact that the urinary excretion of both tamarixetin and isorhamnetin not only originates from the dietary intake, but are also produced as minor endogenous metabolites of quercetin (42).

There was no significant effect of serving size on urinary excretion of the dihydrochalcone phloretin in this crossover analysis. Phloretin is primarily found in apples (27, 39). Our 2 servings diet contained 19.5 g apples, whereas our 5 servings diet contained 48.5 g. Both of these servings represent a low daily intake of apples, which may explain the poor response of phloretin in this study as compared to the previous study by Nielsen et al. (27).

A reliable dose-response calculation between the fruit and vegetable mixture included in the present study and urinary excretion of flavonoids should certainly have included more than three different dose measurements, but nevertheless, the present data seem to indicate that urinary excretion of the flavanones naringenin and hesperetin have a steep and consistent dose-response relationship, whereas urinary excretion of the flavonols appears to exhibit a less pronounced dose dependency (Fig. 1). Still, this more moderate response of the flavonols does not necessarily mean that they are without interest as potential indicators of fruit and vegetable intake.

### Table 4. Effects of crossover intervention with 2 and 5 portions of fruits and vegetables on urinary excretion of flavonoids*

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Period-effect</th>
<th>Period-treatment interaction</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloretin</td>
<td>0.807</td>
<td>0.994</td>
<td>0.082</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>0.848</td>
<td>0.284</td>
<td>0.000</td>
</tr>
<tr>
<td>Naringenin</td>
<td>0.208</td>
<td>0.989</td>
<td>0.000</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>0.769</td>
<td>0.934</td>
<td>0.000</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.742</td>
<td>0.070</td>
<td>0.012</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.058</td>
<td>0.674</td>
<td>0.008</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>0.034</td>
<td>0.011</td>
<td>0.014</td>
</tr>
<tr>
<td>Tamarixetin</td>
<td>0.009</td>
<td>0.275</td>
<td>0.001</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.484</td>
<td>0.777</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Two-sample t tests are used to compare differences in the two groups of participants.

**Period-effect**: Group A (2–5 servings) is tested against Group B (2–5 servings).

**Period-treatment interaction**: Group A (Period 1 + Period 2)/2 is tested against Group B (Period 1 + Period 2)/2.

**Treatment effect**: Group A (Period 1/C0 Period 2) is tested against Group B (Period 1/C0 Period 2).

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**Figure 1.** Twenty-four-hour urinary excretion at baseline, 2 servings, and 5 servings, respectively. *, Different from baseline, $P < 0.05$. **, Different from baseline, $P < 0.001$. #, Different from 2 servings, $P < 0.05$. ##, Different from 2 servings, $P < 0.001$. Columns, medians; bars, 75% percentiles.
intake. Compared with the flavanones, the flavonoids are present in a wider range of fruits and vegetables, and may thus be useful as a more general marker of fruits and vegetables than the former group. As judged from the present study, there seems to be a robust linear relationship between the intake of the fruit and vegetable mixture used in the present study and urinary excretion of quercetin.

Eriodictyol, naringenin and all flavonoids combined seem to demonstrate an almost linear response to serving size increments (Fig. 1). The urinary excretion of hesperetin seems to respond more than expected in the 2–5 servings interval, whereas quercetin seems to respond stronger in the 0–2 servings interval. On the basis of the present results and the results of Nielsen et al. (27), total flavonoids may seem to be a robust flavonoid parameter for mixed fruit and vegetable intake. However, because the concept of "total flavonoids" is poorly defined, it remains to be agreed on what flavonoids should be included in this generic term.

The strongest effect for single flavonoids in the present study was observed for the citrus flavonoids naringenin and hesperetin. In addition, studies have shown that flavonoids are associated with dietary preferences. Flavonoids are a mixture used in the present study and urinary excretion of the flavonoids eriodictyol, naringenin, ingenin and hesperetin, whereas the weakest effect was observed for naringenin and hesperetin, whereas the weakest effect was observed for tamarixetin. Urinary excretion of flavonoids is a promising biomarker for the intake of fruits and vegetables.

Acknowledgments
We thank assistant professor Kerstin Ulla Trygg for her help with hosting our participants, serving, and preparing the food. We thank bioengineers Borghild Arntsen, Anne Randi Alvestad, and Hanne Schulz for collection of blood samples.

References

Lene Frost Anderson, unpublished data.
Urinary Excretion of Flavonoids Reflects Even Small Changes in the Dietary Intake of Fruits and Vegetables


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