



Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status¹⁻³

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ABSTRACT

Background: Epidemiologic studies suggest that foods rich in flavonoids might reduce the risk of cardiovascular disease.

Objective: Our objective was to investigate the effect of intake of flavonoid-containing black currant and apple juice on urinary excretion of quercetin and on markers of oxidative status.

Design: This was a crossover study with 3 doses of juice (750, 1000, and 1500 mL) consumed for 1 wk by 4 women and 1 man corresponding to an intake of 4.8, 6.4, and 9.6 mg quercetin/d.

Results: Urinary excretion of quercetin increased significantly with dose and with time. The fraction excreted in urine was 0.29–0.47%. Plasma quercetin did not change with juice intervention. Plasma ascorbate increased during intervention because of the ascorbate in the juice. Total plasma malondialdehyde decreased with time during the 1500-mL juice intervention, indicating reduced lipid oxidation in plasma. Plasma 2-amino-adipic semialdehyde residues increased with time and dose, indicating a prooxidant effect of the juice, whereas erythrocyte 2-amino-adipic semialdehyde and γ -glutamyl semialdehyde concentrations, Trolox-equivalent antioxidant capacity, and ferric reducing ability of plasma did not change. Glutathione peroxidase activity increased significantly with juice dose.

Conclusions: Urinary excretion of quercetin seemed to be a small but constant function of quercetin intake. Short-term, high intake of black currant and apple juices had a prooxidant effect on plasma proteins and increased glutathione peroxidase activity, whereas lipid oxidation in plasma seemed to decrease. These effects might be related to several components of the juice and cannot be attributed solely to its quercetin content. *Am J Clin Nutr* 1999;69:87–94.

KEY WORDS Quercetin, urinary excretion, biomarker, human intervention, antioxidative status, malondialdehyde, protein oxidation, apple juice, black currant juice, glutathione peroxidase, flavonoids

INTRODUCTION

Results from some epidemiologic studies suggest that a high intake of foods rich in flavonols and flavones might reduce the risk of cardiovascular disease (1–3). The potential beneficial mechanism could be the antioxidative properties of these compounds. Of the dietary plant flavonoids, quercetin and other

flavonols are quantitatively important. The mean intake of quercetin has been estimated at 12 mg/d in Denmark (4) and 16 mg/d in Netherlands (5).

For flavonoids to exert a protective effect on cardiovascular disease it is reasonable to assume that they have to be absorbed. In foods, most flavonoids are present as glycosides, ie, bound to a sugar moiety. Few data are available on absorption of quercetin or quercetin glycosides in humans. The results of a single-dose study by Hollman et al (6), carried out in 9 ileostomy patients, suggested that the absorption of quercetin conjugates from foods may be more efficient than that of the pure aglycone. They observed 52% disappearance in the small intestine after ingestion of 89 mg conjugated quercetin from onions, and a disappearance of only 17% and 24% after ingestion of 100 mg rutinoid or pure quercetin aglycone, respectively. Urinary excretion was 0.31%, 0.07%, and 0.12 % of intake, respectively, for 13 h after ingestion of the dose. In 2 later studies these investigators observed urinary excretion of 0.44% of quercetin from apples and 1.39% from onions over 24 h (7). In an earlier study, Gugler et al (8) detected no urinary excretion with a relatively insensitive analytic method after intake of 4 g quercetin aglycone.

Because quercetin is present in a variety of fruit and vegetables, plasma concentrations or urinary excretion of quercetin may potentially be useful as biomarkers of habitual intake of these foods. To evaluate this potential, information is needed on

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the dose-response and time-response relations. The main aim of the present study was to investigate the dose-response relation between intake and urinary excretion of quercetin when given daily in low doses. A secondary aim was to evaluate whether a fruit juice intervention had an effect on markers of antioxidative status. A 1:1 mixture of apple juice and black currant juice was used as the quercetin source.

SUBJECTS AND METHODS

Study design and diet

The study was a randomized, crossover design with 3 one-week intervention periods separated by ≥ 2 wk. Three doses of fruit juice (black currant and apple juice in a 1:1 mixture) were consumed daily: 750, 1000, and 1500 mL, in the 3 intervention periods, respectively. Subjects were instructed to distribute the juice intake throughout the day in aliquots ≤ 250 mL. Fasting blood samples were collected in the morning of days 1, 2, 3, 5, and 8 in each intervention period, thus reflecting the preceding 24 h; ie, sample day 1 reflects baseline concentrations before intervention (day 0) and sample day 8 reflects the last 24 h of the intervention (day 7). The samples will be referred to in the text according to the 24 h they reflect. Continuous 24-h urine samples were collected at baseline and throughout all intervention periods. The urine samples were collected from 0800 on the listed day of intervention to 0800 the following day. During the intervention periods and during the week preceding an intervention period, the subjects were instructed to exclude flavonoid-containing foods from their diet; tea, wine, spices, and all fruit and vegetables except potatoes were excluded. Food diaries were kept by the subjects during the intervention periods to check their compliance with these instructions. No information on quantitative food intake was available because the diaries were food records by menu without any information on quantities.

Subjects

Five subjects (4 women and 1 man) volunteered for the study. The average age was 25 y (range: 22–28 y) and average body mass index (BMI; in kg/m^2) was 23.3 (range: 21.3–24.9). None of the subjects were pregnant, lactating, or had any chronic illness, and all were nonsmokers. Subjects received oral and written information about the study and gave their written consent. The study was approved by the Research Ethics Committee of Copenhagen and Frederiksberg.

Analysis of ascorbate in juice

The ascorbic acid contents of the apple and black currant juices were determined by HPLC. Samples were extracted with metaphosphoric acid (10% by wt). Before HPLC analysis, samples were filtered through a 0.2- μm filter. Separation of ascorbic acid was performed on 2 PLRP-S columns (250 \times 4.6 mm internal diameter; Polymer Laboratories Ltd, Amherst, MA) at a column temperature of 16°C. The mobile phase was 0.2 mol phosphate buffer/L, pH 2.14, at a flow rate of 0.6 mL/min. Ultraviolet detection was done at 247 nm and ascorbic acid contents were determined by comparison with external standards. The samples were analyzed in duplicate and an in-house control material was included in each analytical series. The lower limit of quantification in juice was 1 mg/L.

Analysis of flavonols in juice and plasma

The flavonol contents of the apple juice, black currant juice, and plasma were determined by HPLC (9). Juice (500 μL) and plasma (300 μL) were diluted to a 2.0-mL solution of 1.2 mol HCl/L in 50% aqueous methanol, with 20 mmol sodium diethyldithiocarbamate/L as an antioxidant. Morin (5 μg) was added with the juices as an internal standard but its use was precluded with plasma because some samples contained a contaminant with a retention time very close to that of morin. The samples were placed in a sealed vial and heated for 2 h at 90°C under continuous stirring with a Recti Therm Heating/stirring Module (Pierce, Rockford, IL). One-fiftieth aliquots of the juice and plasma samples were injected into the HPLC system by using a 4-mm Genesis C-18 cartridge column (150 \times 3.0 mm internal diameter) fitted with a C-18 Genesis guard cartridge (10 \times 4.0 mm internal diameter) in an integrated holder (all from Jones Chromatography, Mid-Glamorgan, United Kingdom). The mobile phase was a 20-min, 20–40% gradient of acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid and eluted at a flow rate of 0.5 mL/min. After ultraviolet detection of the column eluate at 365 nm, postcolumn derivatization was carried out based on procedures described by Hollman et al (10) and fluorescent flavonol complexes were monitored by using an RF-10A fluorometer (Shimadzu Corp, Kyoto, Japan) operating at an excitation wavelength of 420 nm and an emission wavelength of 490 nm. Linear calibration curves were obtained for myricetin, quercetin, morin, and kaempferol between 5 and 250 ng at 365 nm and between 0.1 and 100 ng by using fluorescence detection. Typical recoveries of the morin internal standard added to the juice samples were $>90\%$. Reference compounds were purchased from Sigma Chemicals (Poole, Dorset, United Kingdom).

Collection and preparation of urine samples

Twenty-four-hour urine samples were collected in 2.5-L plastic bottles every day during the intervention periods. In addition, baseline 24-h urine samples were collected the day before the start of the last intervention period. Fifty milliliters 1 mol HCl/L was added to each 2.5-L bottle to increase stability during collection. Urine samples were weighed, their density measured, and pH adjusted to 3–4 with 1 mol HCl/L. Aliquots of 250 mL were stored at -20°C until analyzed.

For analysis of urinary quercetin, a 15-mL aliquot from each 24-h urine sample was enzymatically hydrolyzed to release quercetin from glucuronic acid and sulfate conjugates, and subsequently solid-phase extracted as described previously (11). Briefly, the samples were adjusted to pH 5 with sodium acetate and 1 μg fisetin (Aldrich, Steinheim, Germany) was added as an internal standard. Enzymatic hydrolysis was performed by addition of 100 μL β -glucuronidase/arylsulfatase (*Helix pomatia*, 5.5×10^3 U β -glucuronidase/L, 2.6×10^3 U arylsulfatase/L; Boehringer Mannheim, Mannheim, Germany), and the sample was incubated in a sealed vial for 1 h at 37°C with continuous shaking. After hydrolysis, the sample was solid-phase extracted by using Bond Elut C-18 cartridges (500 mg; Varian, Harbor City, CA). The cartridge was washed with 3 mL 5% methanol, 1% formic acid, and eluted with 3 mL 90% methanol (1% formic acid, 1% ascorbic acid) followed by 3 mL pure methanol. The eluates were combined and evaporated to dryness under vacuum. The residue was dissolved in 150 μL 30% methanol, 1% formic acid, and the whole sample was injected into the HPLC system.



HPLC analyses of urine samples

Quercetin was determined as described by Nielsen and Dragsted (11). In short, the HPLC system consisted of a Hewlett-Packard (Waldbronn, Germany) 1090 system with 3 pumps, 6-port column-switching valve, and diode array detector. Column temperature was maintained constant at 40°C by using a thermostatically controlled column compartment. Detection was carried out simultaneously at 290, 375, and 390 nm, with peak scanning between 210 and 600 nm (2-nm steps) by using the 390-nm signal to quantify the amount of quercetin in the samples. The columns used were a Lichrospher select B, RP-8 (4 × 125 mm, 5 μm, Hewlett-Packard) as column 1 and a Pyrospher RP-18 (4 × 125 mm, 5 μm, Hewlett-Packard) as column 2. The mobile phases used were as follows (flow rate of 1 mL/min): A, 30% methanol:70% 0.05% acetic acid by vol, pH 3.6; B, 23.8 mmol citric acid buffer/L containing 67 μmol EDTA/L, adjusted to pH 2.00 with concentrated phosphoric acid; and C, 100% acetonitrile. Samples were injected into column 1 by using isocratic elution of mobile phase A. By using the automatic 6-port column-switching valve, quercetin and the internal standard fisetin were eluted onto column 2. By gradient elution of mobile phases B and C, quercetin and fisetin were selectively eluted through the ultraviolet detector. Quantification was based on calibration curves achieved from urine samples to which standards of different concentrations had been added. The reproducibility of the method was controlled by analyzing a standard mixture containing 1 μg each of quercetin and fisetin as external standards before and after each series of samples. The limit of detection and quantification of quercetin was 5 μg/L urine, determined by fortifying a quercetin-free urine sample. A control urine sample was included in each series of analyses. All values for this sample were within the range of 20.59–24.70 ± 1.1 μg/L (*n* = 10). Determinations were carried out singly. Recovery of the internal standard fisetin was between 70.2% and 109.9% with an average of 90.1 ± 10% (*n* = 120). If recovery of the internal standard in a single sample was found to be <70% the result was rejected and a new analysis was performed.

Collection of blood samples and separation of erythrocytes

Venous blood samples were taken from subjects in the morning after a minimum of 12 h of fasting and resting supine for 10 min. Subjects were instructed to avoid heavy physical activity for 36 h before blood sampling. Blood samples were collected in EDTA-coated tubes and centrifuged at 1500 × *g* for 10 min at room temperature. Plasma was stored for ≤7 mo at –20°C until analyzed. Samples for plasma ascorbate determination were added to one volume of 10% metaphosphoric acid before freezing. Erythrocytes were washed 3 times with 3 volumes of 0.9% NaCl, resuspended in 1 volume purified water for lysis, and then stored at –80°C until analyzed. Before analysis the lysate was centrifuged at 15000 × *g* (3 min) to remove cell debris. The hemoglobin layer was used for the analyses of antioxidant enzymes.

Antioxidant enzymes

Automated assays were performed on a Cobas Mira analyzer (Roche, Basel, Switzerland) to determine the activity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase in erythrocyte lysates. The activity of the enzymes was related to the amount of hemoglobin in the blood samples. Superoxide dismutase (catalog no. SD 125; Randox, Ardmore, United Kingdom), glutathione per-

oxidase (catalog no. RS 505; Randox), and hemoglobin (catalog no. HG 980; Randox) were determined by using commercially available kits. Glutathione reductase and catalase activities were determined according to methods described by Wheeler et al (12). Glutathione, flavin adenine dinucleotide, purpald, and potassium periodate were purchased from Sigma Chemical Co (St Louis). One erythrocyte sample was selected as a standard and analyzed on 20 different days to obtain a mean and SD. This sample was also analyzed in duplicate with each batch of analyses. The accepted interval for each of these determinations was the established mean ± 2 SDs for each of the 4 enzyme activities; otherwise, the batch was reanalyzed.

Determination of malondialdehyde

Malondialdehyde was determined in plasma by using a thio-barbituric acid–reactive substances (TBARS) HPLC method. The antioxidant butylated hydroxytoluene (BHT) was added to the plasma samples to give a final concentration of 1 mmol/L and alkaline hydrolysis was performed by adding NaOH (final concentration: 0.5 mol/L) and heating for 30 min at 60°C to release any protein-bound malondialdehyde. Reaction with TBARS was performed according to Halliwell and Gutteridge (13), and after the samples were centrifuged for 5 min at 10000 × *g* and 4°C the supernate was transferred to the HPLC analysis system. Samples (40-μL aliquots) were chromatographed with a linear gradient of water and acetonitrile with 0.1% trifluoroacetic acid (0–60%, 17 min). The HPLC analysis was performed on a Hewlett-Packard 1100 system (Waldbronn, Germany) with a diode array detector by using a Pyrospher RP-18 column (4 × 250 mm, 5 μm) with detection at 532 nm. Four malondialdehyde standards were included in each TBARS procedure and HPLC analysis, and the concentration of malondialdehyde in the samples was calculated from a standard curve. The intraday CV for a standard was <3.5% (*n* = 4). Malondialdehyde was purchased from Aldrich Chemical Co, and BHT, 2-thio-barbituric acid, and reduced nicotinamide adenine dinucleotide phosphate were purchased from Sigma Chemical Co. The acetonitrile and methanol used were HPLC grade from Rathburne (Walkerburn, United Kingdom).

Antioxidant capacity

The Trolox (a water-soluble vitamin E analogue)-equivalent antioxidant capacity (TEAC) of plasma was determined by using a commercially available kit (catalog no. NX2332; Randox). The ferric reducing ability of plasma (FRAP) was determined as described by Benzie and Strain (14). The interday CV for a sample analyzed on 5 different days by these tests was 5% for both analyses.

Determination of 2-amino-adipic semialdehyde and γ-glutamyl semialdehyde

Procedures for determination of 2-amino-adipic semialdehyde (AAS) and γ-glutamyl semialdehyde (GGS) were performed as described previously by Daneshvar et al (15). Briefly, the protein sample (1 mg) was dissolved in 0.25 mol *N*-(morpholino)ethanesulfonic acid (MES) buffer/L, pH 6.0, containing 1% sodium dodecyl sulfate and heated for 1 min at 100°C. A solution of 0.25 mol fluoresceinamine/L in 0.52 mol NaOH/L was added, followed by 0.25 mol NaCNBH₃/L in 0.25 mol MES/L, pH 6.0, and the mixture was incubated for 1 h at 37°C. The mixture was applied to a G-25 gel filtration column (Pharmacia, Uppsala, Sweden), and the column eluted with MES buffer. The



protein fraction was collected and precipitated by addition of trichloroacetic acid. The precipitate was hydrolyzed in 6 mol HCl/L at 110°C for 24 h, and an internal standard of acetaldehyde-fluoresceinamine adduct was added. The hydrolysate was filtered and injected into an HPLC apparatus with a Pyrospher RP-18 column (4 × 250 mm, 5 μm) eluted by a linear gradient of 0–50% acetonitrile in 2 mmol formic acid/L, pH 3.2. The eluate was monitored at 454 nm, which is the maximum absorption wavelength of decarboxylated fluoresceinamine, and at 275 nm for determination of free L-tyrosine, which is a marker for the quantity of hydrolyzed protein. The result was corrected for protein hydrolysis and recovery of internal standard. Each sample was analyzed on 2 different days. The variation between determinations of AAS or GGS in each of the plasma and erythrocyte protein samples was not allowed to exceed 10%, otherwise, samples were reanalyzed. Mean intraday variation was <4%.

Determination of plasma ascorbate

Total ascorbic acid content of plasma was determined by a photometric method after oxidation and coupling with 2,4-dinitrophenyl hydrazine (16).

Statistics

Paired comparisons, using Student's *t* test, between the basic and the juice-supplemented diets were performed for all biomarker activities by using the SAS statistical package Proc Means procedure (SAS Institute, Inc, Cary, NC). Multivariate analysis of variance (MANOVA) was performed using the SAS GLM procedure with a repeated statement for the measurements on the same individuals on subsequent days. Correlations were performed by using the GLM procedure.

RESULTS

Dietary intake

The diaries indicated good compliance with the prescribed restrictions: no tea, wine, vegetables, fruit, or spices were consumed during the restricted periods. The most common foods consumed were potatoes, bread and other cereals, meat, fish, and dairy products. Soft drinks, cakes, and sweets were also consumed, but items containing fruit were excluded. Alcoholic drinks other than wine were permitted, except 24 h before collection of blood samples. According to the diaries, beer was the most common alcoholic drink, whereas hard liquor was consumed rarely. Average consumption was <1 drink/d, and ≤3 drinks on any given day. The diaries showed that the foods consumed during the 3 intervention periods were similar for each individual but differed between individuals. In the intervention period with the highest dose, the juice contributed as much as 20–30% of the total estimated energy requirements. The diaries indicated that consumption of snacks (eg, soft drinks, sweets, chocolate, and cookies) was reduced in the intervention periods because these are noted less frequently during periods with juice intake. The short intervention period did not allow estimates of changes in energy balance in the form of changes in body weight.

Contents of flavonoids and ascorbate in the apple and black currant juices

After several commercial juices were screened for their flavonoid content, one apple juice (Nutana, Bjæverskov, Denmark) and one black currant juice (Cadiso, Frederikssund, Den-

mark) were selected for the intervention study on the basis of quercetin content. Both juices were available in grocery stores in Denmark. The quercetin contents of the apple and black currant juices were 6.93 ± 0.57 and 5.87 ± 0.64 mg/L, respectively. The daily intake of quercetin in the 3 intervention periods was 4.8, 6.4, and 9.6 mg/d, respectively. Besides quercetin, the apple juice also contained kaempferol (0.15 ± 0.01 mg/L); the black currant juice contained kaempferol (0.40 ± 0.01 mg/L) and myricetin (4.0 ± 0.1 mg/L).

The ascorbic acid contents of the apple and black currant juices were 5.43 ± 0.10 and 392 ± 3 mg/L, respectively. The high ascorbic acid content of the black currant juice could be ascribed to the addition of ascorbic acid during processing. The daily intake of ascorbic acid from the juices during the 3 intervention periods was 149.0, 198.7, and 298.1 mg/d, respectively.

Urinary and plasma quercetin

Quercetin was detected in all urine samples, but concentrations at baseline were significantly lower than those during intervention ($P < 0.05$ by paired comparison). There was a significant increase in urinary quercetin with dose ($P < 0.0003$) and time ($P < 0.0001$) as tested by MANOVA (Figure 1). The fraction of the quercetin dose excreted into the urine was not dose dependent but increased with time until the third or fourth day of intervention. On average, 0.29% of ingested quercetin was excreted in the urine on day 1 and 0.47% was excreted at steady state (Figure 2). There were no significant interindividual variations in quercetin excretion among the 5 participants ($P > 0.05$, MANOVA). The CV on a single day of intervention was, on average, 28%, but was 15% over a 7-d period. The statistical analyses showed no interactions between the individual quercetin excretion values and the intervention time or dose. The content of quercetin in a control sample and in a urine sample (to which standards of different concentrations had been added) were reproducible within the period of analysis (<3 mo). Plasma samples from day 0 and day 7 were analyzed for quercetin content. However, no significant relation between plasma quercetin con-

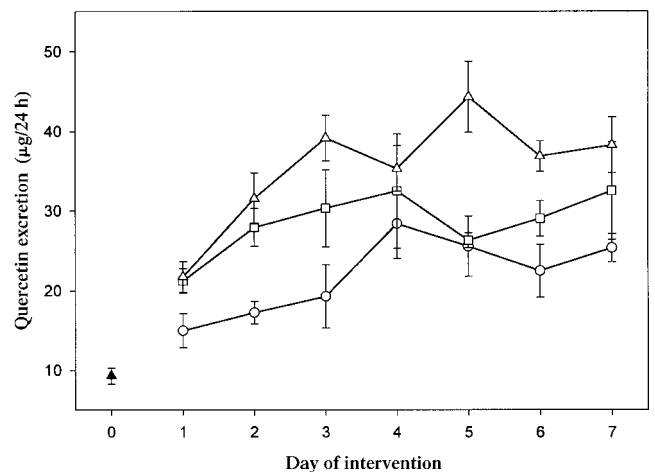


FIGURE 1. Effect of juice consumption on urinary quercetin excretion by juice dose and by day of intervention. Day 0 represents the last day of the basic, low-flavonoid diet before juice intervention started. ▲, control; ○, 750 mL juice (4.8 mg quercetin/d); □, 1000 mL juice (6.4 mg quercetin/d); △, 1500 mL juice (9.6 mg quercetin/d). $\bar{x} \pm$ SEM; $n = 5$.

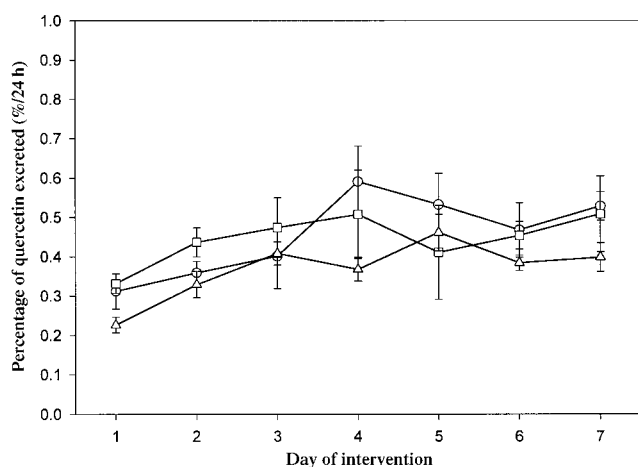


FIGURE 2. Effect of juice consumption on urinary quercetin excretion expressed as a percentage of quercetin content in juice consumed. ○, 750 mL juice (4.8 mg quercetin/d); □, 1000 mL juice (6.4 mg quercetin/d); △, 1500 mL juice (9.6 mg quercetin/d). $\bar{x} \pm \text{SEM}$; $n = 5$.

centration and the juice dose was found (Table 1).

Biomarkers of antioxidative status

The effects of juice consumption on biomarkers of antioxidative status were investigated by comparing blood values for day 0 and day 7 at each dosage (Table 1). There was a significant increase in plasma ascorbate at the highest dose only ($P < 0.01$). Plasma ascorbate concentrations also increased with time of intervention ($P < 0.01$ by MANOVA).

Although a significant decrease in plasma malondialdehyde was observed in the 1500-mL/d intervention period by MANOVA ($P < 0.05$) and also by pairwise comparison with the corresponding baseline values using the t test ($P < 0.05$), there was no significant trend with increasing dose. The overall decrease in malondialdehyde with duration of intervention was

not significant by MANOVA. There were differences between the 3 intervention periods for malondialdehyde, but correction for this did not change the outcome of the statistical analysis. A graphic representation of the variation in malondialdehyde with dose and time is shown in Figure 3. A decreasing trend in plasma malondialdehyde with duration seemed to be restricted to the 750- and 1500-mL/d intervention periods. An overall significant decrease with duration of juice intervention was observed for TEAC by MANOVA ($P < 0.02$). However, the decrease was only significant on day 7 at the intermediate dose ($P = 0.05$) by paired comparison with the values before intervention. No significant change in FRAP was observed with dose or with duration of intervention. AAS in plasma proteins increased after 1000 or 1500 mL/d juice ($P < 0.01$). The increase in AAS was found to be both time- ($P < 0.001$) and dose dependent ($P = 0.05$) as determined by MANOVA. Differences were also observed between the 3 intervention periods, but corrections for interperiod differences did not change the significant increases with time and dose. A graphic representation is shown in Figure 4. No change in either AAS or GGS in erythrocyte proteins was observed, however. Glutathione reductase, superoxide dismutase, and catalase activities increased at the 2 lower doses, and the increase was significant at the 1000-mL/d dose; however, a decrease was observed at the highest dose, which was significant in the cases of superoxide dismutase and catalase. Consequently, there were no significant overall changes in the activities of these enzymes with juice dose as determined by MANOVA. For glutathione peroxidase there were significant differences between periods and a correction was made by normalizing values according to mean baseline values (day 0). There was an overall increase in glutathione peroxidase activity with dose, and the increase was significant at the 2 higher doses. There were apparent overall increases in activities of glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase with duration of dosing, but they were not significant as determined by MANOVA ($P = 0.85$, $P = 0.40$, $P = 0.08$, and $P = 0.10$, respectively).

TABLE 1

Effects of juice consumption on plasma ascorbate, plasma quercetin, and biomarkers of antioxidative status¹

Biomarker	Basic diet ²	Change with juice		
		750 mL	1000 mL	1500 mL
Plasma				
Ascorbate ($\mu\text{mol/L}$)	65.7 \pm 13.3	19.0 \pm 20.4	19.1 \pm 22.5	33.2 \pm 13.7 ³
Quercetin ($\mu\text{g/L}$)	14.9 \pm 9.9	0.4 \pm 3.1	16.8 \pm 34.5	-6.1 \pm 15.7
AAS (pmol/mg protein)	29.6 \pm 2.8	8.3 \pm 11.3	9.6 \pm 4.1 ³	18.2 \pm 8.5 ³
Malondialdehyde (pmol/mg protein)	78.5 \pm 7.5	-11.6 \pm 10.3	0.67 \pm 5.4	-11.6 \pm 8.9 ⁴
TEAC (mmol/L)	1.67 \pm 0.05	-0.32 \pm 0.71	-0.68 \pm 0.83 ⁴	-0.41 \pm 0.76
FRAP (nmol/L)	736 \pm 162	-29 \pm 46	-25 \pm 90	25 \pm 175
Erythrocyte				
AAS (pmol/mg protein)	33.0 \pm 3.1	0.4 \pm 7.6	3.1 \pm 9.4	-2.5 \pm 17.0
GGS (pmol/mg protein)	35.3 \pm 3.2	-1.4 \pm 6.0	4.4 \pm 5.8	2.4 \pm 2.7
Glutathione reductase (U/g hemoglobin)	8.49 \pm 0.92	0.64 \pm 0.57	0.43 \pm 0.34 ⁴	-0.44 \pm 0.77
Glutathione peroxidase (U/g hemoglobin)	60.4 \pm 10.8	2.5 \pm 5.0	3.4 \pm 1.7 ⁴	5.6 \pm 4.3 ⁴
Superoxide dismutase (U/g hemoglobin)	997 \pm 97	49 \pm 59	75 \pm 58 ⁴	-108 \pm 48 ³
Catalase (U/g hemoglobin)	13.0 \pm 1.92	0.89 \pm 0.62 ⁴	3.2 \pm 0.54 ⁵	-2.8 \pm 1.7 ⁴

¹ $\bar{x} \pm \text{SD}$; $n = 5$. AAS, 2-amino-adipic semialdehyde; TEAC, Trolox-equivalent antioxidant capacity; FRAP, ferric reducing ability of plasma; GGS, γ -glutamyl semialdehyde.

²Subjects' habitual diet without tea, wine, spices, vegetables, and fruit.

³⁻⁵Significantly different on day 7 from basic diet (two-tailed pairwise t test): ³ $P < 0.01$, ⁴ $P < 0.05$, ⁵ $P < 0.001$.

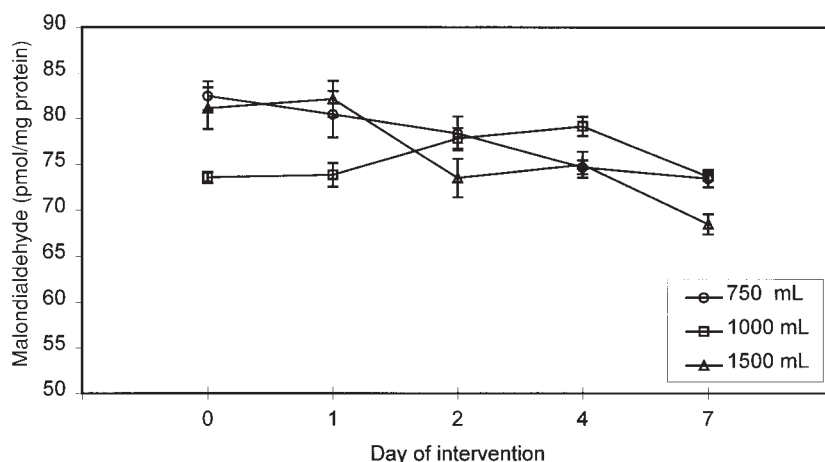


FIGURE 3. Effect of juice consumption on plasma malondialdehyde by juice dose and by day of intervention. Day 0 represents the last day of the basic, low-flavonoid diet before juice intervention started. Values are corrected for differences between the means of the 3 intervention periods. Significant decrease in malondialdehyde with juice intake for the highest dose only, $P < 0.05$ (by MANOVA). $\bar{x} \pm \text{SEM}$; $n = 5$.

DISCUSSION

Urinary quercetin excretion

As far as we know, this study is the first to investigate excretion of quercetin during a low-dose intake period of several days. In contrast, previous studies have been based on the administration of a single large dose, 70–100 mg (6, 7, 17). Only a small fraction, 0.29–0.47%, of quercetin intake was excreted in urine. This agrees with results reported from a single-dose study with 100 mg quercetin from apples (0.44%), but is lower than the fraction of quercetin excreted from fried onions (1.39%) for 24 h after ingestion of the dose (7). Similarly, low excretion (<0.37%) of another flavonoid, naringenin, was reported after ingestion of grapefruit juice coadministered with verapamil (18), whereas others have observed much higher excretion of naringenin and hesperitin: 6.8% and 24.4%, respectively, after grapefruit juice and orange juice intervention (19). High excretions,

≈21% and 9%, respectively, have also been observed for 2 isoflavonoids found in soymilk, daidzein and genistein (20). In accordance with what we observed for quercetin in the present study, the excreted fraction of the isoflavonoids remained constant at 3 different doses (20).

The fraction of quercetin intake excreted in urine reached steady state after 3–4 d of intervention, indicating an elimination half-life of ≈24 h. This agrees well with results from a single-dose study with 100 mg quercetin glycoside from an apple preparation with an average elimination half-life from plasma of 23 h (7).

Interindividual variation in urinary excretion of quercetin was nonsignificant, as tested by MANOVA. However, when based on only 1 d of excretion, the interindividual CV was almost twice as high as when based on 7 d (28% compared with 15%). Similar interindividual variation in 24-h quercetin excretion was observed by Hollman et al (7) in 9 participants after a single dose of onions or apples.

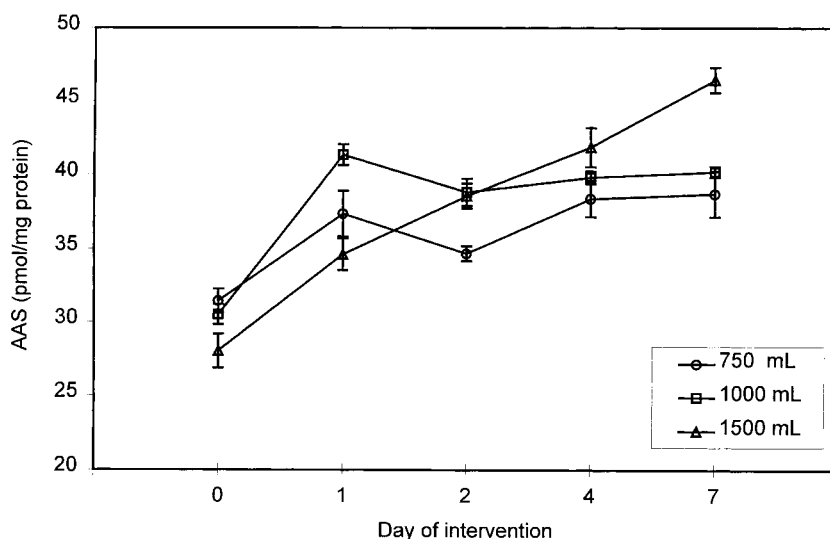


FIGURE 4. Effect of juice consumption on plasma 2-amino-adipic semialdehyde (AAS) by juice dose and by day of intervention. Day 0 represents the last day of the basic, low-flavonoid diet before juice intervention started. Values are corrected for differences between the means of the 3 intervention periods. Significant increases by duration of juice intervention, $P < 0.001$, and juice dose, $P = 0.05$. $\bar{x} \pm \text{SEM}$; $n = 5$.

Urinary quercetin was detected in all urine samples, even the baseline samples collected after the participants had avoided flavonoid-containing foods for 1 wk. The baseline concentration was low and could originate either from a low quercetin intake from foods other than fruit, vegetables, spices, tea, and wine (which were omitted), or from residual excretion of dietary quercetin ingested before the flavonoid-free diet was instituted. Assuming a 24-h elimination half-life of quercetin, the latter would point to a relatively high intake of flavonoids (≈ 70 mg/d) before the basic diet was instituted.

The presence of quercetin in urine shows that it was absorbed by the gut, but the urinary content does not necessarily reflect absolute absorptive efficiency because absorbed quercetin may be metabolized, stored, or excreted through other routes such as the biliary tract. However, the results from the present study suggest that urinary quercetin may be a useful biomarker of quercetin intake because it fulfills 2 key criteria for such a marker: a constant fraction excreted, independent of dose, and a nonsignificant interindividual variation in response. If further investigations confirm these results, the quercetin content of 24-h urine samples could be used to estimate intake despite the small fraction excreted by this route. Such a biomarker would be most valuable, for epidemiologic studies in particular, because all calculations of intake are linked to a large error due to the limited information on the quercetin content of foods and to the substantial variation in the quercetin content of certain foods.

In apples and black currants, as in other foods, quercetin exists in the form of glycosides, for which the sugar moiety and its position may be important for absorption. The identity of the quercetin glycosides in the 2 juices used here was not established. Large differences in absorption between main dietary sources of quercetin would make it difficult to use urinary quercetin excretion as a biomarker of total quercetin intake because the excreted fraction depends on the absorbed fraction (7). In that case, urinary quercetin might be useful as a marker of absorbed quercetin, and such a marker might be even more relevant than a marker of total quercetin intake.

Quercetin in plasma

The low quercetin concentrations in fasting blood samples and the lack of a dose response suggest that plasma quercetin concentrations may be relatively insensitive as a biomarker of quercetin intake. Similarly, low plasma concentrations of the isoflavones daidzein and genistein were observed 24 h after ingestion of soymilk (20). Plasma concentrations of naringin (18, 19) and hesperitin (19) below quantification limits were reported after grapefruit (18) and grapefruit and orange (19) juice ingestion.

Biomarkers of antioxidative status

When evaluating the effect of the present intervention study on markers of antioxidative status, bear in mind that the apple and black currant juices selected for the present study contained not only the flavonoids quercetin, myricetin, and kaempferol but also several other phenolic antioxidants, including simple phenolic acids, chalcones, and anthocyanins (21, 22). They also contained ascorbic acid, which resulted in a significant increase in plasma ascorbate during intervention.

It has been suggested that the antioxidant properties of dietary flavonoids explain their inverse association with cardiovascular disease observed in some epidemiologic studies (1–3), although


this association was not observed in others (23, 24). Our finding of a decrease in total plasma malondialdehyde after an intake of 1500 mL juice/d over 7 d suggests an improvement in antioxidant status and indicates that even low amounts of flavonoids or other antioxidant constituents of the juice might decrease lipid oxidation within the plasma compartment. Therefore, a direct antioxidant action of these constituents on plasma lipoproteins could be one mechanism that reduces risk for cardiovascular disease. We also observed a significant difference between the 3 intervention periods but baseline values did not change significantly, so carryover effects do not seem to be responsible. The antioxidative effect of juice consumption was not as clearly observable by measurements of TEAC or FRAP. Although TEAC decreased significantly overall, the effect was only significant at the intermediate dose. Our finding of a significantly increased glutathione peroxidase activity with dose might indicate an effect of the juice intervention on this activity. The other antioxidant enzymes in the erythrocytes did not show a consistent pattern of change but increased at lower doses and decreased at the highest dose. One week may seem to be a short period for expression of an enzyme increase in erythrocytes, which lack the capability for protein synthesis, but consistent short-term effects, even within hours, on some of these enzymes have been reported previously (25).

The strong prooxidant action of juice consumption on plasma lysine residues seems to be directly related to the juice intervention. Because the effect was immediate, dose related, and related to the duration of intake, it cannot be merely a chance finding. The increase was observed in all 5 participants and in all 3 intervention periods. The significant differences between the intervention periods were not reflected in significant differences at baseline, indicating that interactions between the periods were not responsible. AAS was shown previously to respond well to oxidative stress in rats exposed to acrolein or to *tert*-butylhydroperoxide (15) and to be related to oxidative stress in humans from exposure to traffic exhaust (26). We questioned the participants in the present study about their exposure to traffic exhaust during work time, transportation, or during sports activities and seemed to rule out any relation between our findings and traffic exhaust exposure (data not shown). Our observation of an increase in the oxidative damage to serum albumin after juice consumption suggests the presence of potentially prooxidative compounds in the juice. In vitro studies have shown prooxidant as well as antioxidant behavior of flavonoids (27). We are currently trying to confirm the effect of juice on AAS in animal experiments to identify potentially harmful compounds in the juice.

The results for AAS compared with those for malondialdehyde in the present study illustrate that even within plasma, there are several subcompartments that may respond differently to a dietary challenge. These results also contradict a general pro- or antioxidant state in the blood compartment but indicate a differential protection or damage to specific structures, depending on their interaction with the dietary components reaching them. However, the enzyme activity results and those for other markers of antioxidant status need to be confirmed and extended in long-term studies.

In conclusion, quercetin from apple and black currant juice was absorbed and 0.29–0.47% of intake was excreted in urine. A high juice intake seemed to have a prooxidant effect on plasma proteins, whereas a putative marker of lipid oxidation, malondialdehyde, in plasma, seemed to decrease. Glutathione peroxi-



dase, a marker of antioxidative defense, also increased with juice dose. These effects might be related to several components of the juice and cannot be attributed solely to its quercetin content. 

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