Plasma Kinetics and Urinary Excretion of the Flavanones Naringenin and Hesperetin in Humans after Ingestion of Orange Juice and Grapefruit Juice[1]

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ABSTRACT The flavanones naringenin and hesperetin exhibit estrogenic, anticarcinogenic and antioxidative properties. Orange juice and grapefruit juice contain high amounts of these compounds, and therefore their intake from the diet can be relatively high. No data are available regarding plasma concentrations or plasma kinetics of flavanones. The objectives of this study were to develop methods allowing the analysis of naringenin and hesperetin from plasma and urine and to study their plasma kinetics and urinary excretion. We also wanted to assess whether plasma or urine flavanone concentrations can be used as biomarkers of intake. Healthy volunteers ingested orange juice (five women and three men) or grapefruit juice (two women and three men) once (8 mL/kg). Eleven blood samples and urine were collected between 0 and 24 h after juice administration. Flavanones were analyzed by HPLC with electrochemical detection. Naringenin and hesperetin were bioavailable from the studied juices, but interindividual variation in bioavailability was remarkable. The resulting plasma concentrations were comparatively high, and the peak plasma concentrations (C_{max}) were 0.6 ± 0.4 μmol/L (means ± SD) for naringenin from orange juice and 6.0 ± 5.4 μmol/L for naringenin from grapefruit juice. The corresponding value for hesperetin from orange juice was 2.2 ± 1.6 μmol/L. The elimination half-lives were between 1.3 and 2.2 h, and therefore plasma concentrations reflect short-term intake. The relative urinary excretion varied depending on the flavanone source and dose and was 30.2 ± 25.5% and 1.1 ± 0.8% for naringenin from grapefruit juice and orange juice, respectively, and 5.3 ± 3.1% for hesperetin from orange juice. The considerable difference in the relative urinary excretion of naringenin from the two juices was most likely caused by dose-dependent renal clearance rather than differences in bioavailability (as indicated by the similar C_{max}-to-dose ratios). The results indicate that urine flavanone concentrations are not good biomarkers of dietary intake. We conclude that because of the relatively high concentrations of flavanones in plasma after ingestion of orange juice or grapefruit juice, considerable health effects could ensue in individuals consuming them regularly. J. Nutr. 131: 235–241, 2001.

KEY WORDS: • *flavonoids* • *naringenin* • *hesperetin* • *kinetics* • *bioavailability* • *humans*

It has been recognized for many years that a diet rich in vegetables and fruits protects against chronic diseases. The role that individual compounds play in this protection has been a subject of much research. Currently, there is a great interest in phenolic compounds called flavonoids. Several epidemiological studies have suggested a protective effect of these compounds on cardiovascular diseases (Hertog et al. 1993 and 1995, Knekt et al. 1996, Yochum et al. 1999). However, research on flavonoids has mainly focused on two subgroups of flavonoids: the flavonols and the isoflavones. The flavanones, also called citrus flavonoids, on the other hand, have received less attention, although their intake from the diet can be quite high and they exhibit promising biological activities.

Epidemiological studies indicate a protective relationship between the consumption of citrus fruits or juices and the risk of ischemic stroke (Joshipura et al. 1999) and lung cancer (Le Marchand et al. 1999). In the United States, the mean daily individual consumption of citrus fruits and juices has been estimated as 68 g, of which 59 g was consumed as juices (U.S. Department of Agriculture 1997). The most commonly used citrus juices (i.e., orange and grapefruit juices) contain high amounts of the flavanones hesperetin and naringenin (Kawaii et al. 1999, Mouly et al. 1998). In Finland, the average intakes have been estimated as 8.3 mg naringenin and 28.3 mg hesperetin per d (Kumpulainen et al. 1999). These values correspond to 15 and 51%, respectively, of the total flavonoid intake.

Naringenin and hesperetin are phytoestrogens, which could affect sex hormone–mediated biological responses by several different mechanisms, including binding to estrogen receptors (Dechaud et al. 1999, Huang et al. 1997, Hunter et al. 1999, Kuiper et al. 1998, Ruh et al. 1995). The compounds also possess anticarcinogenic (So et al. 1996, Tanaka et al. 1997, Yang et al. 1997), antioxidant (van Acker et al. 2000) and blood lipid–lowering (Bok et al. 1999, Borradaile et al. 1999, Santos et al. 1999, Shin et al. 1999) activities. In addition,

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[3] Abbreviations used: AUC_{0-24}, area under the plasma concentration–time curve from 0–24 h; CL_{ren}, renal clearance; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}, $T_{1/2}$, terminal half-life.

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naringenin is an inhibitor of cytochrome P450 enzymes (Ghosal et al. 1996), and for this reason, its role in the clinically relevant drug-grapefruit interactions has been studied extensively (Fuhr 1998, Ubeaud et al. 1999).

Flavonoids are usually present in plants as glycosides (i.e., bound to different sugars). The main flavonoid glycosides of oranges are hesperidin (hesperetin-7-rutinoside) and narirutin (naringenin-7-rutinoside) (Kawaii et al. 1999). Naringin (naringenin-7-neohesperidoside) and, to a lesser extent, narirutin are the predominant flavonoids of grapefruit. Little information about the absorption or the kinetic behavior of flavanones is available, but studies concerning urinary excretion of flavanones have shown that the compounds are bioavailable and are excreted, at least to some extent, into the urine (Ameer et al. 1996, Fuhr and Kummert 1995, Lee and Reidenberg 1998). Several studies also indicate that the flavanone glycosides are hydrolyzed in the gastrointestinal tract before absorption of the aglycones (Choudhury et al. 1999, Fuhr and Kummert 1995, Jang and Kim 1996). To date, no data are available on plasma naringenin and hesperetin concentrations or their plasma kinetics after ingestion of the compounds either as pure substances or in foods that contain them.

The objectives of the present study were to develop analytical methods allowing the measurement of naringenin and hesperetin concentrations in plasma and urine and to characterize the absorption, the plasma kinetics and the urinary excretion of naringenin and hesperetin after the intake of orange juice and grapefruit juice.

MATERIALS AND METHODS

Subjects. Eight volunteers (five women and three men) were recruited into the orange juice study. Subject characteristics (means ± SD) were weight of 73 ± 15 kg (range 50–95 kg), body mass index of 23 ± 3 kg/m^2 (range 19–28 kg/m^2) and age of 26 ± 5 y (range 20–34 y). Five volunteers (two women and three men) were recruited into the grapefruit study. Subject characteristics (means ± SD) were weight of 71 ± 15 kg (range 50–90 kg), body mass index of 23 ± 3 kg/m^2 (range 18–26 kg/m^2) and age of 32 ± 3.3 y (range 28–37 y). One subject participated in both studies (referred to as subject 2 in Fig. 2); the other subjects participated in only one of the studies.

The subjects were all apparently healthy with no history of disease of the gastrointestinal tract, such as lactose intolerance or irritable bowel syndrome. None of the subjects were taking medications, except for three women in the orange juice study who used oral contraceptives. The subjects followed a citrus-free diet for 1 wk before the study and during the study day. The subjects were given oral instructions on the diet and a list of prohibited foods, which included all foods and beverages known or suspected to contain citrus ingredients. The subjects were also asked to restrain from using dietary supplements during this period. The study protocol was approved by the Ethics Committee of the National Public Health Institute (KTL). Informed written consent was obtained from all volunteers before participation in the study.

Study design. The orange juice study and the grapefruit study were performed on separate days. In both studies, the amount of ingested juice was 8 mL/kg. The ingested amounts ranged between 400 and 760 mL of orange juice and between 400 and 720 mL of grapefruit juice. The subjects ingested the juice within 7 min at the study site in the morning after an overnight fast. The subjects were allowed to eat for the first time 4 h after ingestion of the test juice. Baseline urine and blood samples were obtained 10–20 min before juice administration. Blood samples were collected into vacuum tubes containing EDTA at 1, 2, 3, 4, 6, 8, 10, 12, 14 and 24 h after the juice was consumed. Urine was collected into plastic bottles in four fractions (0–4, 4–8, 8–14 and 14–24 h) for 24 h. All urine was collected, and the participants were instructed to empty their bladder before starting to collect a new fraction. The urine bottles were stored at 4°C. The amount of urine in each fraction was measured, and urine samples were frozen at −70°C immediately after the participants had started to collect the next fraction. Blood samples were centrifuged at 1000 × *g*, and plasma was frozen at −70°C within 30 min after a blood sample was taken.

The subjects stayed at the study site until the 14-h samplings, after which they went home to sleep, and they returned to the study site the next morning for the last samplings. Therefore, for the larger part of the study, the study personnel were able to check that the participants followed the instructions regarding urine collection and diet. However, the participants were allowed to go out for walks or to work in separate rooms at the study site between samplings. Compliance with the 1-wk citrus-free diet was checked from a questionnaire, which the participants filled out during the study day. According to the questionnaires, only a few minor deviations occurred during the first days of the 1-wk citrus-free diet. Accordingly, none of the participants had measurable levels of flavanones in plasma or urine at baseline.

Citrus juices. The juices used in this study were obtained from local supermarkets and were chilled juices manufactured from concentrate. The orange juice had been produced from oranges (*Citrus sinensis*) of the cultivar Pera, and the grapefruit juice had been manufactured from grapefruit (*Citrus paradisi*) of the cultivars white March (99%) and white Duncan (1%). Their trade names are Valio Appelsiini Täysmehu and Valio Greippi Täysmehu (Valio Ltd., Helsinki, Finland).

Reagents and chemicals. The naringenin and hesperetin standards were obtained from Sigma Chemical Co. (St. Louis, MO). The suppliers of other reagents and chemicals have been reported earlier (Erlund et al. 2000).

Analytical methods. The concentrations of naringenin and hesperetin in plasma, urine and juices were analyzed using analytical methods developed at our laboratory. With these methods, the samples are hydrolyzed enzymatically, and the analytes are extracted from proteins before analysis by HPLC. The enzyme used cleaves conjugates, such as glucuronic acids, sulfates and sugars from the aglycone. Therefore, the results presented in this report represent total naringenin and hesperetin concentrations, which include unconjugated flavanones, flavanones conjugated with glucuronic acid, sulfate or glycoside groups and flavanones either bound or not bound to protein.

Hesperetin and naringenin conjugates were hydrolyzed by incubating 0.5 mL of EDTA plasma or 0.5 mL of urine with 55 μL of 0.78 mol of sodium acetate buffer (pH 4.8)/L, 50 μL of 0.1 mol ascorbic acid/L and 20 μL of a crude preparation from *Helix pomatia* (type HP-2; Sigma Chemical Co.) for 17 h at 37°C. Each sample was diluted with 2 mL of phosphate buffer (70 mmol/L, pH 2.4) and added to a Bond Elut C18 solid phase extraction column, preconditioned with 6 mL of methanol and 6 mL of phosphate buffer. The column was washed with 9 mL of phosphate buffer and 0.5 mL of water. Flavanones from plasma samples were eluted with 4 mL of methanol and dried. After this, 300 μL of methanol and 100 μL of 5.3 mol of acetic acid/32 mmol of oxalic acid (80:20, v/v) (pH 2.4) per L were added. The tubes were centrifuged for 15 min at 2000 × *g*, and the clear liquid was transferred into 300-μL HPLC vials for HPLC analysis. Flavanones from urine samples were eluted with 4.5 mL of methanol, and 1.5 mL of 5.3 mol acetic acid and 32 mmol oxalic acid (80:20, v/v) (pH 2.4) per L were added to the methanol eluate. This extract was used for HPLC analysis.

For extraction of flavanones from juices, 150 μL of orange juice or grapefruit juice, 1 mL of H_2O, 110 μL of 0.78 mol sodium acetate buffer (pH 4.8)/L, 100 μL of 0.1 mol ascorbic acid/L and 200 μL of a crude preparation from *H. pomatia* (type HP-2; Sigma Chemical Co.) were incubated for 17 h at 37°C. The flavanones were extracted as described for plasma samples, except that the final 400-μL methanol-acid mixture was diluted 1:100 before HPLC analysis.

Chromatographic analysis was performed with a system consisting of an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA), a Coulochem 5100A electrochemical detector with a model 5011 analytical cell (ESA, Chelmsford, MA) and an Inertsil ODS-3 analytical HPLC column (250 × 4.0 mm i.d., 5 μm) (GL Sciences, Tokyo, Japan).

For the analysis of naringenin from all matrices, the mobile phase consisted of 40% of acetonitrile in phosphate buffer (70 mmol/L, pH 2.4). The first cell of the electrochemical detector was set to 500 mV,

and the second cell was set to 700 mV. For the analysis of hesperetin, the mobile phase was 58% methanol in phosphate buffer (70 mmol/L, pH 2.4). The first cell of the electrochemical detector was set to 350 mV, and the second cell was set to 550 mV. In all HPLC analyses, the flow rate of the eluent was 1 mL/min and the injection volume was 30 μL.

Quantification of the naringenin and hesperetin peaks from all matrices was based on the standard additions method. For the analysis of naringenin from plasma, for instance, plasma standards containing 0.00, 0.12, 0.23, 0.46, 0.92, 1.83 and 3.67 μmol of added naringenin/L were prepared. The standards were treated exactly the same way as samples (i.e., they contained the same ingredients as actual samples, and they were subjected to hydrolysis and extraction procedures). Therefore, the results could be read directly from the standard curve, and no corrections due to recovery losses and so on had to be made. The standard curves were obtained by plotting the peak height of standards versus flavanone concentration.

Day-to-day variation (CV %) for both naringenin and hesperetin from all matrices was <10%, and within-day variation was <6%. Recovery of naringenin and hesperetin from all matrices was 70–80%. The limit of detection for naringenin and hesperetin from plasma was 74 and 33 nmol/L, respectively. The linearity of the method for naringenin in plasma and urine in the concentration ranges 0.11–3.67 and 0.27–276.00 μmol/L were $y = 1.4x - 15.5$ ($r^2 = 0.999$) and $y = 18.6x + 4.8$ ($r^2 = 0.999$), respectively. The linearity of the method for hesperetin in plasma and urine in the concentration ranges of 0.03–4.97 and 0.06–248.00 μmol/L were $y = 9.5x + 3.5$ ($r^2 = 0.999$) and $y = 29.7x + 3.7$ ($r^2 = 0.999$), respectively.

Calculation of pharmacokinetic indexes and statistical analysis. The pharmacokinetic indexes were calculated by model-independent methods. The peak plasma concentration (C_{max}) and the time to reach it (T_{max}) were taken directly from the data. The elimination half-life ($T_{1/2}$) was calculated from the equation $T_{1/2} = \ln 2/k$, using the terminal monoexponential log-linear slope of the time-vs- concentration curve of each subject for the estimation of k by the least-squares method. The area under the plasma concentration–time curve (AUC_{0-24}) was calculated using the trapezoidal method. CL_{ren} was obtained by dividing the total amount of flavanone excreted in the urine in 24 h by AUC_{0-24}. All data are expressed as means ± SD.

Differences between the means of selected pharmacokinetic indexes (T_{max}, $T_{1/2}$, CL_{ren}, relative urinary excretion and C_{max}-to-ingested dose ratio) for naringenin from grapefruit and naringenin from orange juice were tested by Mann-Whitney's U test. A P-value of <0.05 was considered significant. The means of the pharmacokinetic indexes of hesperetin were not statistically compared with the corresponding indexes of naringenin because although both of the compounds are flavanones, they are nevertheless different chemical compounds. Spearman's correlation was used to study the association between plasma flavanone AUC_{0-24} and relative urinary excretion values. This was done separately for each flavanone.

RESULTS

Flavanone concentrations of the citrus juices. The naringenin and hesperetin glycosides present in the juices were hydrolyzed to the aglycones naringenin and hesperetin before quantification by HPLC. The concentrations of naringenin and hesperetin in the orange juice were 151 μmol/L (41 mg/L) and 722 μmol/L (218 mg/L), respectively. The concentration of naringenin in the grapefruit juice was 1283 μmol/L (349 mg/L).

Pharmacokinetics of naringenin after ingestion of orange juice and grapefruit juice. Naringenin was absorbed by all subjects from both the orange juice and the grapefruit juice, but there were great interindividual differences in the AUC_{0-24} and C_{max} values. The C_{max} values were 0.1–1.2 μmol/L for naringenin from orange juice and 0.7–14.8 μmol/L for naringenin from grapefruit juice. None of the subjects had measurable concentrations of naringenin in plasma at baseline. The mean and individual time-vs.-plasma concentration curves for naringenin from both juices are shown in **Figs. 1** and **2**. The pharmacokinetic indexes for naringenin are shown in **Table 1**.

Pharmacokinetics of hesperetin after ingestion of orange juice. Hesperetin was absorbed from orange juice by all subjects. The kinetic behavior of hesperetin was similar to that of naringenin, and interindividual variations in the AUC_{0-24} and C_{max} values were also remarkable for hesperetin. The C_{max} values for hesperetin were 0.5–5.5 μmol/L. None of the subjects had measurable hesperetin concentrations in plasma at baseline. The mean and individual time-vs.-plasma concentration curves for hesperetin are shown in Figs. 1 and 2. The pharmacokinetic indexes calculated from the data are shown in Table 1. Because negligible amounts of hesperetin glycosides are present in grapefruit juice (Kawaii et al. 1999), plasma hesperetin concentrations were measured only after ingestion of orange juice.

Urinary excretion and renal clearance (CL_{ren}) of flavanones. The cumulative urinary excretion curves for the flavanones are shown in **Fig. 3**. Both flavanones were mainly excreted into the 4- to 8-h fraction after ingestion of the two juices. The percentage excreted into the 4- to 8-h fraction of the total excreted amount was 83 and 63% for naringenin from orange juice and grapefruit juice, respectively. The corresponding value for hesperetin was 65%.

Values for total urinary excretion, relative urinary excretion as a percentage of intake and CL_{ren} are shown in Table 1. The relative urinary excretions of naringenin from grapefruit and orange juices were 30.2 and 1.1%, respectively. The corresponding value for hesperetin from orange juice was 5.3%. The clearance of the flavanones increased with increasing dose, and the differences were statistically significant (Table 1). Variations in clearance were small within the groups.

Correlation between plasma AUC_{0-24} values of flavanones and relative urinary excretion. Strong correlations between plasma flavanone AUC_{0-24} and relative urinary excretion values were found for naringenin from grapefruit ($r_s = 0.91$, $P < 0.05$) and for hesperetin from orange juice ($r_s = 0.976$, $P < 0.01$). For naringenin from orange juice, there was no correlation ($r_s = 0.571$, $P > 0.05$).

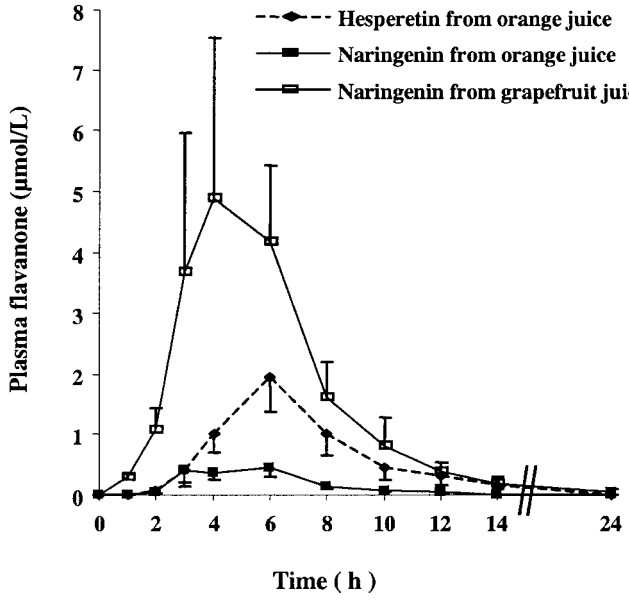

FIGURE 1 Plasma naringenin and hesperetin concentrations in healthy men and women after single ingestions (8 mL/kg) of orange juice ($n = 8$) or grapefruit juice ($n = 5$). Values are means ± SEM.

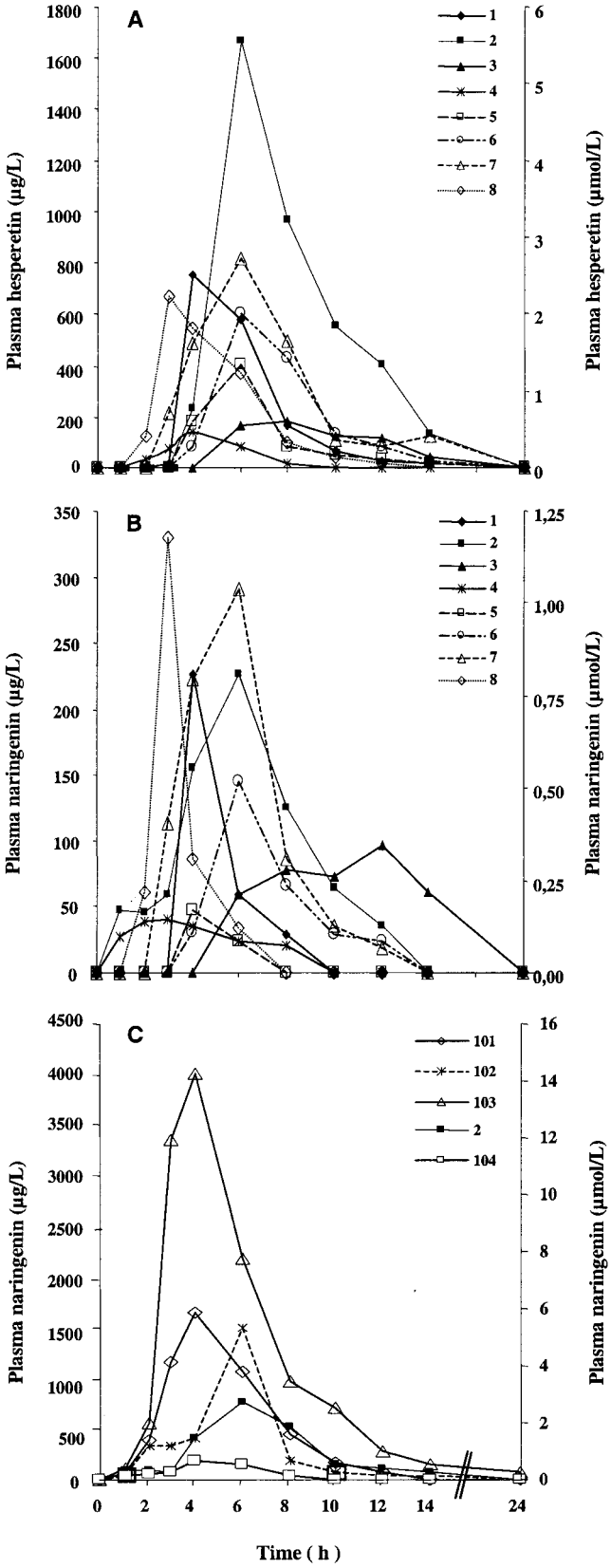

FIGURE 2 Individual plasma concentration curves for hesperetin after ingestion of orange juice (*A*) and naringenin after ingestion of orange juice (*B*) or grapefruit juice (*C*). The men and women ingested 8 mL of either orange juice or grapefruit juice per kg once. Subject numbers are shown.

TABLE 1

Pharmacokinetic indexes for naringenin and hesperetin after single ingestion (8 mL/kg) of orange juice (n = 8) or grapefruit juice (n = 5) by men and women[1]

	Naringenin[2]		Hesperetin[3]
	Grapefruit juice	Orange juice	Orange juice
Ingested dose of flavanone			
µmol	731 ± 155	85 ± 7	417 ± 86
mg	199 ± 42	23 ± 2	126 ± 26
AUC_{0-24}			
µmol · h/L	27.7 ± 26.3	2.6 ± 1.6	10.3 ± 8.2
µg · h/L	7534 ± 7151	719 ± 437	3099 ± 2464
C_{max}			
µmol/L	5.99 ± 5.36	0.64 ± 0.40	2.20 ± 1.58
µg/L	1628 ± 1459	175 ± 110	655 ± 479
T_{max},[4] *h*	4.8 ± 1.1	5.5 ± 2.9	5.4 ± 1.6
$T_{1/2}$,[4] *h*	2.2 ± 0.1[a]	1.3 ± 0.6[a]	2.2 ± 0.8
Renal clearance,[4] *L/h*	8.4 ± 1.5[b]	0.4 ± 0.2[b]	2.4 ± 0.5
Total urinary excretion			
µmol	223.0 ± 180.0	1.1 ± 0.87	22.3 ± 16.7
mg	60.78 ± 48.99	0.29 ± 0.24	6.74 ± 5.06
Relative urinary excretion,[4]			
% of intake	30.2 ± 25.5[c]	1.1 ± 0.8[c]	5.3 ± 3.1
C_{max}-to-ingested dose ratio ($\times 10^{-3}$),[4] L^{-1}	8.1 ± 7.4	7.7 ± 4.9	5.2 ± 3.2

[1] Values are means ± SD.
[2] Naringenin was obtained from both orange juice and grapefruit juice.
[3] Hesperetin was obtained from orange juice.
[4] Comparisons between means of selected pharmacokinetic indexes for naringenin from orange juice and naringenin from grapefruit juice by Mann-Whitney *U* test: [a] $P < 0.05$, [b] $P < 0.002$, [c] $P < 0.002$.

DISCUSSION

The rationale for performing the study was to establish whether flavanones from orange and grapefruit juices are bioavailable and to evaluate whether their plasma concentrations are high enough and their kinetic behavior such that an effect on human health could be expected. We also wanted to assess whether plasma or urine flavanone concentrations can be used as biomarkers of their intake. For these purposes, we developed analytical methods that allow for the first time the analysis of hesperetin and naringenin from plasma. The methods are reproducible, sensitive and suitable for the measurement of flavanones from urine as well. HPLC with electrochemical detection was used because of its superior selectivity toward these compounds compared with UV detection.

There has been some controversy regarding how flavonoids are absorbed, and it has been suggested that some flavonoids, mainly those with glucose molecules as their sugar side chains, might be absorbed intact (Hollman et al. 1999). However, flavonoids such as rutin, hesperidin, naringin and narirutin, which contain rutinoses or neohesperidoses, are most likely hydrolyzed by intestinal enzymes of bacterial origin, such as α-rhamnosidases and β-glucosidases, before absorption of the aglycones (Bokkenheuser et al. 1987, Choudhury et al. 1999, Erlund et al. 2000, Fuhr and Kummert 1995, Hollman et al. 1999, Jang and Kim 1996).

Naringenin and hesperetin were absorbed from orange juice and naringenin was absorbed from grapefruit juice by all subjects. There were, however, great interindividual variations in the bioavailability of the compounds as indicated by the great

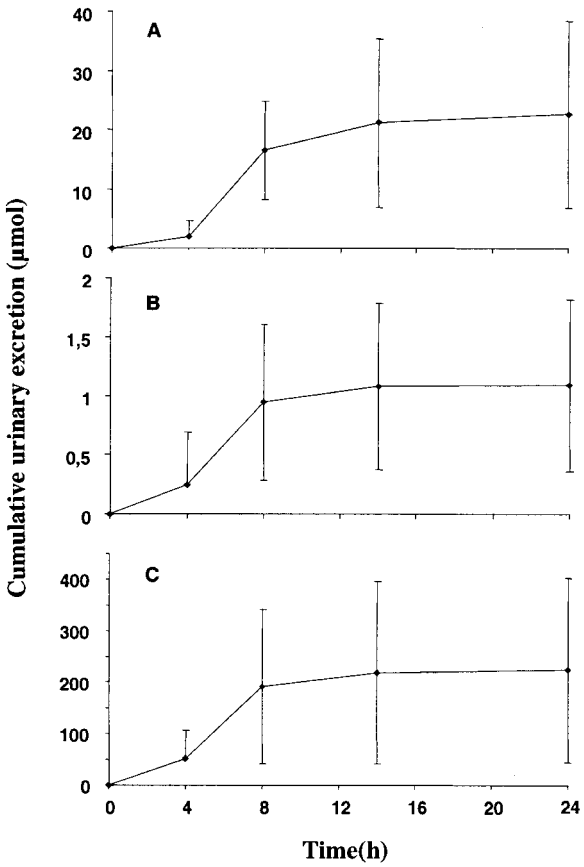

FIGURE 3 Cumulative urinary excretion curves of men and women for hesperetin from orange juice (*A*), naringenin from orange juice (*B*) and naringenin from grapefruit juice (*C*). The subjects ingested 8 mL of either orange juice ($n = 8$) or grapefruit juice ($n = 5$) per kg once. Values are means ± SD.

variation in the C_{max} and AUC values in subjects receiving the same relative dose. We hypothesize that these variations were caused by differences in gastrointestinal microflora. In this study, peak plasma concentrations of naringenin and hesperetin were reached between 4.8 and 5.5 h, which indicates that naringenin and hesperetin from orange and grapefruit juices are absorbed from the distal parts of the small intestine or the colon, where, as mentioned earlier, enzymes capable of cleaving the flavonoid glycosides in question are present. In some subjects, there were measurable concentrations of flavanones in plasma as quickly as 1 h after ingestion of a juice, which might seem to be too short a time for a compound to reach the ileum or the colon. However, large amounts of liquid taken in the fasting state transit the gastrointestinal tract much more quickly than a normal meal. It is also possible that there were small amounts of other glycosides present in the juices, such as naringenin 7-*O*-glucoside in grapefruit juice (Castillo et al. 1993), which could have been absorbed more quickly than the main flavanone glycosides. The results of this study are similar to those of previous reports on quercetin-rutinoside regarding T_{max} (Erlund et al. 2000, Hollman et al. 1999) and interindividual variation in bioavailability (Erlund et al. 2000). Large interindividual variation has also been reported to occur for isoflavones (Rowland et al. 2000).

In this study, the relative urinary excretion (as a percentage of intake) of flavanones varied greatly depending on the source from which they were obtained. The variation was most likely caused by dose-dependent CL_{ren} and not, or at least not entirely, by differences in bioavailability. The fact that the C_{max}-to-ingested dose ratio was almost equal for naringenin from grapefruit juice and orange juice strongly suggests that absorption was not saturated at these doses. On the other hand, differences in absorption efficiency cannot be ruled out because the rutinosides and the neohesperosides could be cleaved by different enzymes, or they could be cleaved by the same enzymes with different affinities for the different flavanone glycosides. Dose-dependent CL_{ren} can occur for several reasons (Rowland and Tozer 1995). For vitamin C, for instance, an active renal reabsorption mechanism becomes saturated at high plasma concentrations, which results in faster CL_{ren} (Kallner et al. 1979). Saturation of plasma proteins can also cause faster clearance because compounds not bound to plasma proteins are more readily excreted into the urine. At these flavanone concentrations, complete saturation of plasma proteins is not likely, but if the fraction of unbound flavanones increases with increasing plasma concentration, urinary excretion could be enhanced at higher doses. In general, CL_{ren} can also be affected by urine flow or urine pH. In this study, no correlation was found between these factors and CL_{ren} (data not shown).

Our results indicate that in addition to renal excretion, other routes of elimination are involved. This view is supported by the fact that no correlation between the individual AUC_{0-24} values for naringenin and the relative urinary excretion of the compound was found after ingestion of orange juice, although such a correlation was found for both hesperetin from orange juice and naringenin from grapefruit juice. In other words, when the intake of flavanone was high, there was a correlation between AUC_{0-24} and urinary excretion, but when the ingested dose was small, there was no correlation. Also, although the CL_{ren} of naringenin from grapefruit juice was considerably faster than it was for naringenin from orange juice, the $T_{1/2}$ was significantly smaller for the compound from the latter source. The $T_{1/2}$ of a compound would be expected to decrease, not increase, with increasing clearance. Other possible routes of elimination are metabolism and biliary excretion. An efficient but saturable hepatic mechanism causing biliary excretion could account for the lower $T_{1/2}$ of naringenin from orange juice.

On the whole, our results regarding urinary excretion of flavanones are similar to those of previous reports (Ameer et al. 1996, Fuhr and Kummert 1995, Lee and Reidenberg 1998). Based on urinary data, the $T_{1/2}$ of naringenin and naringenin glucuronides after ingestion of grapefruit juice was estimated as 2.9 and 2.6 h, respectively (Fuhr and Kummert 1995), which is similar to our results. The relative urinary excretions of naringenin and hesperetin after ingestion of 1250 mL orange juice and 1250 mL grapefruit juice, on the other hand, were estimated as 6.8 and 24.4%, respectively (Ameer et al. 1996), which is quite different from our results. The pharmacokinetics of hesperetin has not been previously studied, and our report is the first on the subject.

When interpreting the pharmacokinetic results of this study, it should be kept in mind that they could have been different if pure compounds had been used. Other compounds present in the juices could affect the mechanisms involved with the absorption, disposition and elimination of the studied flavanones. Furthermore, it is important to realize that the plasma curves would look different if the juices were given together with a meal. Flavanones would most likely be detected in plasma for a longer period of time. The C_{max} values would be lower, but the AUC_{0-24} values would not necessarily be smaller.

One of our main focuses is to study the association between intake of nutrients and chronic diseases by developing plasma or urine biomarkers that reflect dietary intake and to link these to disease data. The results of this study indicate that the CL_{ren} of the flavanones varies depending on the dose and that the use of urine flavanone concentrations as biomarkers of their dietary intake is therefore problematic. Because of the short half-life of the compounds, plasma flavanone concentrations probably also do not reflect long-term intake. If plasma naringenin or hesperetin concentrations are used to study the association between flavanones and diseases, the results should be complemented with nutritional intake data obtained through dietary assessment methods. The nutritional approach is supported by the assumption that people who drink citrus juices probably do so quite regularly and are capable of estimating their consumption fairly accurately. On the other hand, the dietary assessment methods do not take into account the great interindividual variation in the bioavailability of the flavanones. Because the sources of error differ for these two approaches, the errors can be minimized by the combined use of both methods. The situation is quite different for naringenin and hesperetin than for the flavonol quercetin. We (Erlund et al. 2000) and others (de Vries et al. 1998) have previously shown that plasma concentrations of quercetin can be used as a biomarker of its intake. Linking together plasma concentrations with disease data are a better way of studying the quercetin-disease associations than the nutritional approach, because accurate estimation of the daily intake of the most important source of quercetin (i.e., onions) is extremely difficult and because interindividual variation in the bioavailability of quercetin from quercetin-rutinoside, the major flavonol of tea, is remarkable.

In summary, the results of this study show that naringenin and hesperetin are bioavailable from orange juice and grapefruit juice and that interindividual variation in bioavailability is considerable. Plasma hesperetin and naringenin concentrations are comparatively high after the ingestion of orange and grapefruit juices. Thus, we conclude that if the biological activities ascribed to these flavanones actually prevail in vivo, considerable health effects could ensue in the large group of individuals consuming orange or grapefruit products on a regular basis.

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