Flavanone plasma pharmacokinetics from blood orange juice in human subjects

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Some blood orange juice (BOJ) flavanones may have antioxidant, anti-inflammatory, anti-allergic, hypolipidaemic, vasoprotective and anticarcinogenic properties. The aim of the present study was to evaluate the pharmacokinetics of hesperetin and naringenin in human subjects after BOJ intake. In a cross-over study, seven healthy female volunteers consumed 150 and 300 ml BOJ corresponding to about 51–102 mg hesperetin and to 6–12 mg naringenin, respectively. Plasma samples were collected before, each hour for 8 h and 24 h after BOJ administration and analysed for their content of hesperetin and naringenin by liquid chromatography–MS/MS. The plasma concentrations of these compounds were dose dependent and the peak concentration (C_{max}) was reached in 5·1 (SD 0·6) h after BOJ intake. The C_{max} of hesperetin was 43·4 (SD 32·4) and 79·8 (SD 60·1) ng/ml after 150 and 300 ml intake, respectively. For naringenin the plasma peak was 16·4 (SD 11·9) and 34·0 (SD 20·6) ng/ml. Moreover, the conjugated forms of these flavanones represent more than 95 % of the plasma concentration. The results indicate that both hesperetin and naringenin are bioavailable after BOJ intake; naringenin seemingly more so than hesperetin.

Flavanones: Blood orange juice: Pharmacokinetics: Human studies

Flavonoids are important micronutrients present in the human diet, and in the past decade an increasing number of studies regarding the positive effects on human health of these natural compounds have been reported (Le March, 2002; Morrissey & Watson, 2003; Manach *et al.* 2005*a*; Scalbert *et al.* 2005). Good sources of these compounds are citrus fruit juices obtained from oranges and grapefruit. These fruits and their beverages contain mainly the flavanones hesperidin (hesperetin-7-rutinoside) and narirutin (naringenin-7-rutinoside) (Tomas-Barberan & Clifford, 2000) and lesser amounts of anthocyanins and carotenoids when the juice is obtained from blood oranges.

The hesperidin and narirutin ingested with the food are metabolised by human intestinal bacterial microflora to the aglycones hesperetin and naringenin, respectively (Choudhury *et al.* 1999; Felgines *et al.* 2000; Matsumoto *et al.* 2004).

These compounds possess antioxidant (Heo *et al.* 2004; Hirata *et al.* 2005), anticarcinogenic (Shen *et al.* 2004), hepatolipidaemic (Kurowska & Manthey, 2004) and anti-inflammatory (Rotelli *et al.* 2003) activities. In addition, naringenin and hesperetin are phyto-oestrogens. In fact, like isoflavones, they can bind to oestrogen receptors (Harris *et al.* 2005), or inhibit aromatase activity, the rate-limiting enzyme in the conversion of androgens to oestrogens (Edmunds *et al.* 2005).

Recently, a study was carried out (Riso *et al.* 2005) supplementing human volunteers for 21 d with blood orange juice (BOJ) to evaluate its effect on plasma antioxidant concentrations and on DNA damage and lipid peroxidation. The results revealed an improvement of lymphocyte DNA resistance to oxidative stress after BOJ intake. Despite these well-documented effects on human health the information about naringenin and hesperidin bioavailability in human subjects after BOJ intake is poor. In fact, the pharmacokinetics of these flavanones has been studied using mainly juices obtained from blond oranges and grapefruits (Erlund *et al.* 2001; Manach *et al.* 2003). Moreover, these studies were performed using high amounts of juice or pure compounds (Kanaze *et al.* 2006).

The aim of the present study was to evaluate by liquid chromatography (LC)–MS/MS the plasma pharmacokinetics of naringenin and hesperetin after the intake of low amounts of BOJ.

Subjects and methods

Chemicals

Naringin, narirutin, hesperidin, didymin, morin, naringenin and hesperetin were from Extrasynthese (Genay, France). β-Glucuronidase–sulfatase from *Helix pomatia* was purchased from Sigma (St Louis, MO, USA). Methanol, acetonitrile, sodium and ammonium acetate were from Merck (Darmstadt, Germany). Water was obtained from a MilliQ apparatus (Millipore, Milford, MA, USA). The squeezed and pasteurised BOJ from organic culture was obtained

from Fattoria Scaldasole (Monguzzo, Italy). The orange juice had been produced from oranges (*Citrus sinensis*) of the cultivars Tarocco and Sanguinello. The BOJ used was portioned in 500 ml containers which were stored at $-20°C$. After the containers were thawed, the juices were immediately consumed.

Subject and study design

Healthy female subjects (*n* 7; age $25·7 ± 1·1$ years; BMI $16·2–21·8 kg/m^2$) were selected after completing a questionnaire concerning their dietary habits and lifestyle. They were not taking any supplement, drug or medication. Informal, written consent was obtained from each participant and the local ethical committee approved the protocol. Volunteers refrained from consuming citrus fruits or juices for 1 d before the study. On the day of the experiment subjects were confined to the laboratory. Between 08.30 and 09.00 hours the fasted volunteers received 150 and 300 ml of BOJ in random order on two occasions 1 month apart. Lunch consisted of bread (70 g), cheese (20 g), ham (50 g), ice-cream (80 g) and decaffeinated coffee (one cup). The dinner consisted of pasta (80 g), olive oil (10 g), parmigiano cheese (15 g), meat (100 g), bread (70 g) and decaffeinated coffee (one cup). After dinner (8 h after BOJ intake), the volunteers were allowed to leave the laboratory, returning the next morning for the final blood sampling. Blood samples were collected in tubes containing lithium-heparin before, each hour for 8 h and 24 h after BOJ administration. Time zero was obtained 30 min before the juice ingestion.

Flavanone determination in blood orange juice

BOJ was diluted 10-fold in methanol:1 % formic acid (90:10, v/v) and the resulting solution was sonicated for 10 min and centrifuged at $1000 g$ for 10 min. One sample of the supernatant fraction was filtered through a Millipore $0·2 μm$ disk; $5 μl$ were used for analysis. The chromatographic system consisted of an Alliance model 2695 (Waters, Milford, MA, USA) equipped with a model 2996 (Waters) photodiode array detector and a triple quadrupole mass spectrometer model Quattro micro (Micromass, Beverly, MA, USA). A $3·5 μm$ C_{18} Symmetry column ($150 × 2·1 mm$; Waters) was used for the separation, which was performed by means of a linear gradient elution (eluent A, $0·1 %$ formic acid; eluent B, acetonitrile) at a flow rate of $250 μl/min$. The column was maintained at 30°C. The gradient was as follows: from 10 to 30 % B in 20 min and then from 30 to 60 % B in 10 min. Chromatographic data were acquired in the 200–450 nm range and were integrated at 290 nm. The mass spectrometer operated in full-scan mode in the range 100–1000 Da. All data were acquired by Masslink 4.0 software (Micromass). The flavanone concentrations were evaluated in six different BOJ containers, and each analysis was carried out in triplicate.

Calibration curves were obtained from narirutin, naringin, didymin, hesperidin, naringenin and hesperetin stock solutions prepared by dissolving 5 mg of standard powder in 5 ml methanol. They were measured in the range of $2–100 μg/ml$.

Analysis of hesperetin and naringenin in plasma

Hesperetin and naringenin conjugates were hydrolysed by incubating $100 μl$ heparinised plasma with $50 μl$ internal standard (I.S.) (morin; 100 ng/ml) in $0·1 M$-CH_3COONa buffer (pH 5·2) and $100 μl$ glucuronidase–sulfatase ($1 U/μl$) at 37°C for 18 h. The reaction mixture was extracted with $500 μl$ ethylacetate, vortexed and centrifuged at $1000 g$ for 1 min. The supernatant fraction ($400 μl$) was dried under N_2 and the residue dissolved in $100 μl$ methanol. To evaluate free hesperetin and naringenin, a plasma sample ($100 μl$) was incubated with $50 μl$ I.S. and $100 μl$ of $0·1 M$-CH_3COONa buffer (pH 5·2) for 18 h at 37°C. The reaction mixture was then treated as described earlier.

The HPLC system was an Alliance 2695 (Waters) coupled to a Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray source operating in negative mode. The analytical column was a $3·5 μm$ Symmetry ($150 × 2·1 mm$) and the flow rate was fixed to $250 μl/min$. The eluent was $2 mM$-CH_3COONH_4 (pH 5):acetonitrile (70:30, v/v). The injected volume was $10 μl$. The capillary voltage was set to $3·0 kV$, the cone voltage was 40 V, the source temperature was 130°C, and the desolvating temperature was 350°C. The collision energy was 35 eV, and Argon 6·0 (Sapio, Monza, Italy) was used at $2·2 × 10^{-3}$ mbar to improve fragmentation in the collision cell. Naringenin and hesperetin plasma levels were determined in multiple reaction-monitoring mode. The calibration curves were in the range of $2–100 ng/ml$ and the detection limit was 0·5 and 1 ng/ml for naringenin and hesperetin, respectively.

Assessment of matrix effect on flavanone assay in plasma

The assessment of a matrix effect was evaluated as described by Matuszewski *et al.* (2003) Briefly, three sets of five standard lines (2, 10, 25, 50 and 100 ng/ml) were prepared to evaluate the assay accuracy, precision, recovery, and absence or presence of a matrix effect. The first set was prepared to evaluate the MS/MS response for naringenin, hesperetin and I.S. injected in the mobile phase. The second set was prepared in plasma extracts spiked after extraction. The third set was prepared in plasma spiked before extraction. The matrix effect on the quantification of flavanones and I.S. was assessed by comparing the absolute areas, peak areas ratios, and slopes of the standard lines between these three different sets. In addition, precision and accuracy of the method and recovery of analytes were also determined.

Statistical analysis

Statistical analyses were performed with the STATISTICA software (Statsoft Inc., Tulsa, OK, USA). A two-way repeated-measures ANOVA with the amount of juice and the time as the independent factor was used. The peak plasma concentration (C_{max}) and the time taken to reach peak concentrations are expressed as mean values and standard deviations.

The elimination rate constant (K) was determined in the terminal phase from the slope of the regression line of the concentrations in plasma samples. Three time points, starting with the time taken to reach peak concentration, were included in

the estimation of K. The terminal half-life was calculated as $0.6935/K$. Area under the curve (AUC) was calculated in the range 0–24 h, although between 8 and 24 h there was no plasma sample, according to the following equation:

$$\text{Area} = (1/2)(C_0 + C_1)(t_1 - t_0) + (1/2)(C_1 + C_2)(t_2 - t_1)\dots$$
$$+ (1/2)(C_8 + C_{24})(t_{24} - t_8),$$

where C is flavanone concentration and t denotes time.

Results

The content of flavanones in blood orange juice

Fig. 1 shows a typical LC–diode-array detection MS (LC–DAD-MS) chromatogram of BOJ. Narirutin and hesperidin eluted at about 16.3 and 17.9 min, respectively. Their identity was established by co-chromatography, 'on-line' UV spectra comparison and molecular ion evaluation. Peak 3 shows a UV spectrum suggesting a structure of a flavanone derivative. MS of peak 3 yielded a negative ion at m/z 593, and a product ion at m/z 285, suggesting that it may contain an aglycone moiety. Aglycones (peaks 5 and 6) were not present in BOJ. The content of hesperidin and narirutin in BOJ was 68.6 (SD 1.9) and 8.2 (SD 0.6) mg/100 ml, respectively. The peak 3 concentration was 10.3 (SD 0.5) mg/100 ml. Naringin was not found in the analysed BOJ. Thus, the ingestion of 150 or 300 ml of BOJ provided 51.3 (SD 0.9) or 102.4 (SD 1.8) mg hesperetin and 5.9 (SD 0.2) or 11.8 (SD 0.4) mg naringenin, respectively. The detection limit was about 1 μg/ml for both hesperidin and narirutin. The overall reproducibility of quantitative analysis of narirutin and hesperidin was 2.3 (SD 0.2) and 3.2 (SD 0.4) % for intra- and inter-day analysis, respectively. Moreover, BOJ flavanones were stable over 6 months when stored at −20°C in a closed brik.

Analysis of flavanones in plasma

Under the applied conditions, naringenin and hesperetin were separated in less than 13 min, and their plasma concentrations calculated by external standardisation from calibration curves. Hesperetin and naringenin plasma levels were evaluated after hydrolysis of samples by β-glucuronidase–sulfatase. The conjugated forms of these flavanones represent more than 95 % of the plasma forms. None of the subjects had detectable amount of naringenin or hesperetin at baseline.

Fig. 2 shows an example of naringenin and hesperetin peaks detected by LC–MS/MS in an enzymically hydrolysed plasma sample obtained after 0 and 5 h of BOJ intake. Mean naringenin and hesperetin plasma levels following the intake of 150 and 300 ml BOJ are reported in Table 1. ANOVA showed a significant effect of time ($P < 0.0001$) and the amount of BOJ consumed on the absorption of naringenin ($P = 0.022$) and hesperetin ($P = 0.006$).

Fig. 3 shows naringenin and hesperetin plasma levels in all subjects after BOJ intake. The compounds were absorbed by all subjects but there were great inter-individual differences in the AUC$_{(0-24\,h)}$ and C_{max} values. Flavanones at baseline were not detected. The highest plasma level (C_{max}) of naringenin and hesperetin was reached 5–6 h after the BOJ ingestion; these levels decreased significantly after 8 h. At 24 h after BOJ intake flavanones were no longer detectable in plasma.

The C_{max} of hesperetin was 43.4 (SD 32.4) and 79.8 (SD 60.1) ng/ml after 150 and 300 ml BOJ intake, respectively. For naringenin the peak plasma was 16.4 (SD 11.9) and 34.0 (SD 20.6) ng/ml. Table 2 summarises the individual and group mean values of the kinetics indexes calculated from the flavanone plasma levels and expressed as apparent pharmacokinetics measured as AUC and dose adjusted (AUC/dose). In particular, after the 300 ml portion, significantly higher levels of hesperetin and naringenin were observed after 5 and 4 h, respectively, with respect to the 150 ml portion.

The hesperetin half-life was 1.1 (SD 0.5) and 1.0 (SD 0.5) h for 150 and 300 ml BOJ intake, respectively. For naringenin the half-life was 1.3 (SD 1.0) and 0.9 (SD 0.4) h for 150 ml and 300 ml of BOJ intake, respectively.

Percentage recovery of flavanones from plasma

The recovery of the extraction for hesperetin, naringenin and I.S. from spiked plasma samples was 94.2 (SD 3.2), 96.1 (SD 3.3) and 85 (SD 2.4) %, respectively. The precision of the method was tested by both intra-day (n 6) and inter-day (5 d; n 6) reproducibility, and the CV was below 8 %.

Discussion

The aim of the present study was to establish hesperetin and naringenin pharmacokinetics after BOJ intake. To this end, we developed mass spectrometric methods for the analysis of hesperetin and naringenin in plasma sample and their glycosides in BOJ. In the present work, the negative ion electrospray ionisation mass spectrum of BOJ showed abundant ion peaks at $(m/z)^-$ 579 and $(m/z)^-$ 609, corresponding to narirutin and hesperidin, respectively. In BOJ, hesperidin and narirutin were the main flavanones present and their amount was estimated to be about 686 and 82 mg/l, respectively. Moreover, a more apolar compound (peak 3; Fig. 1) was found. LC–electrospray ionisation-MS analysis of this unknown compound yielded an intense peak at $(m/z)^-$ 593. MS/MS analysis of the $(m/z)^-$ 593 ion as precursor ion gave a product ion with $(m/z)^-$ 285. The mass data, combined with the UV and chromatographic behaviour, suggest that this peak is didymin (Robards et al. 1997). Peak identity was then confirmed by authentic standard.

From our data, a moderate BOJ intake (300 ml) provides about 128 mg flavanones. At this time no data regarding total flavonoid intake in Italy are available. In the USA the dietary polyphenol intake has been estimated to be about 1 g/d (Kuhnau, 1976). Several subsequent studies have provided additional data concerning the intake of various classes of polyphenols (Justesen et al. 1997; Sampson et al. 2002), and the dietary flavonoid intake in Western countries is now estimated to be in the range 100–150 mg/d (Manach et al. 2004). In Italy, the only available data on flavonoid intake concerns the flavonols and has been estimated as about 35 mg/d (Pietta et al. 1996). The intake of 300 ml BOJ, consequently, provides a significant amount of flavonoids, and can play a role against oxidative stress.
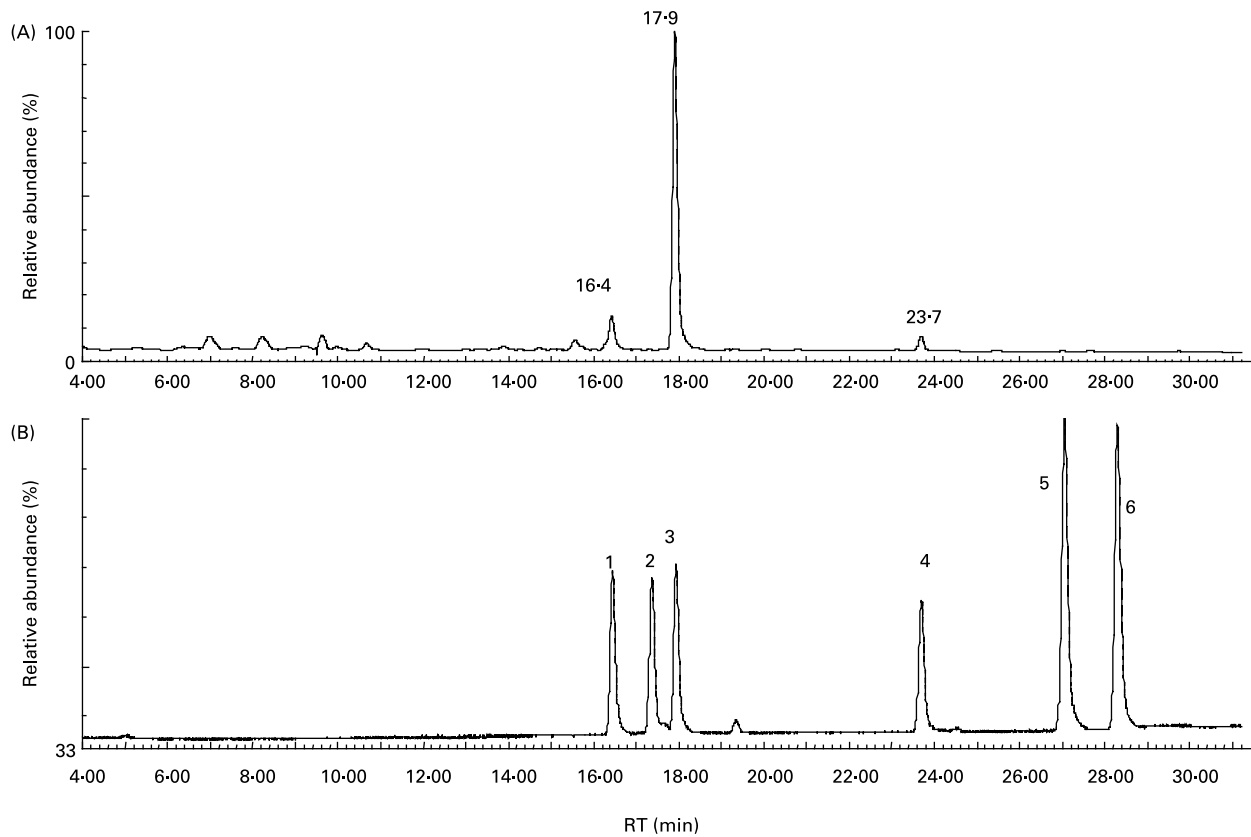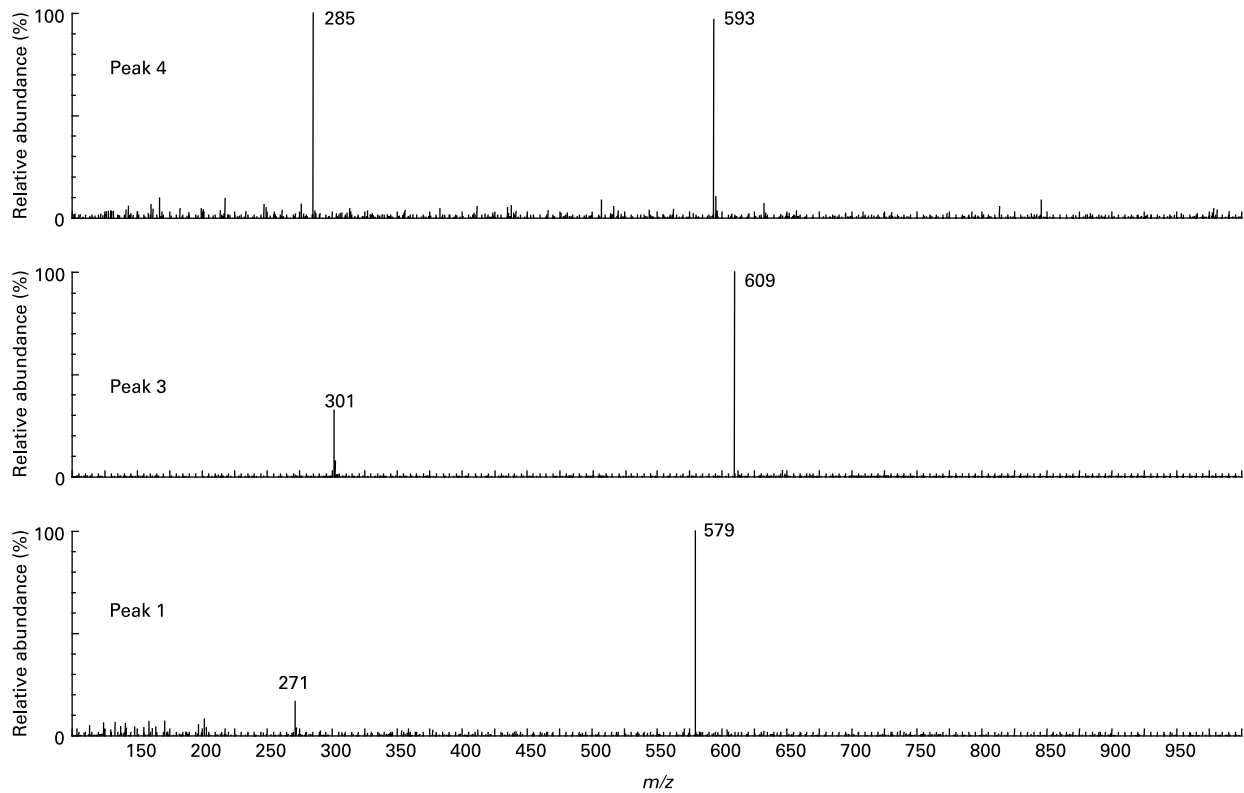
Fig. 1. Liquid chromatography−diode-array detection MS (LC-DAD-MS) chromatograms of blood orange juice (A) and standard solutions (B) at 290 nm. Peak 1 represents narirutin (retention time (RT) 16·4 min, $(m/z)^-$ 579, 271 (aglycone). Peak 2 represents naringin (RT 17·2 min, not present in blood orange juice). Peak 3 represents hesperidin (RT 17·9 min, $(m/z)^-$ 609, 301 (aglycone)). Peak 4 represents didymin (RT 23·7 min, $(m/z)^-$ 593, 285 (ion product)). Peak 5 represents naringenin; peak 6 represents hesperetin.
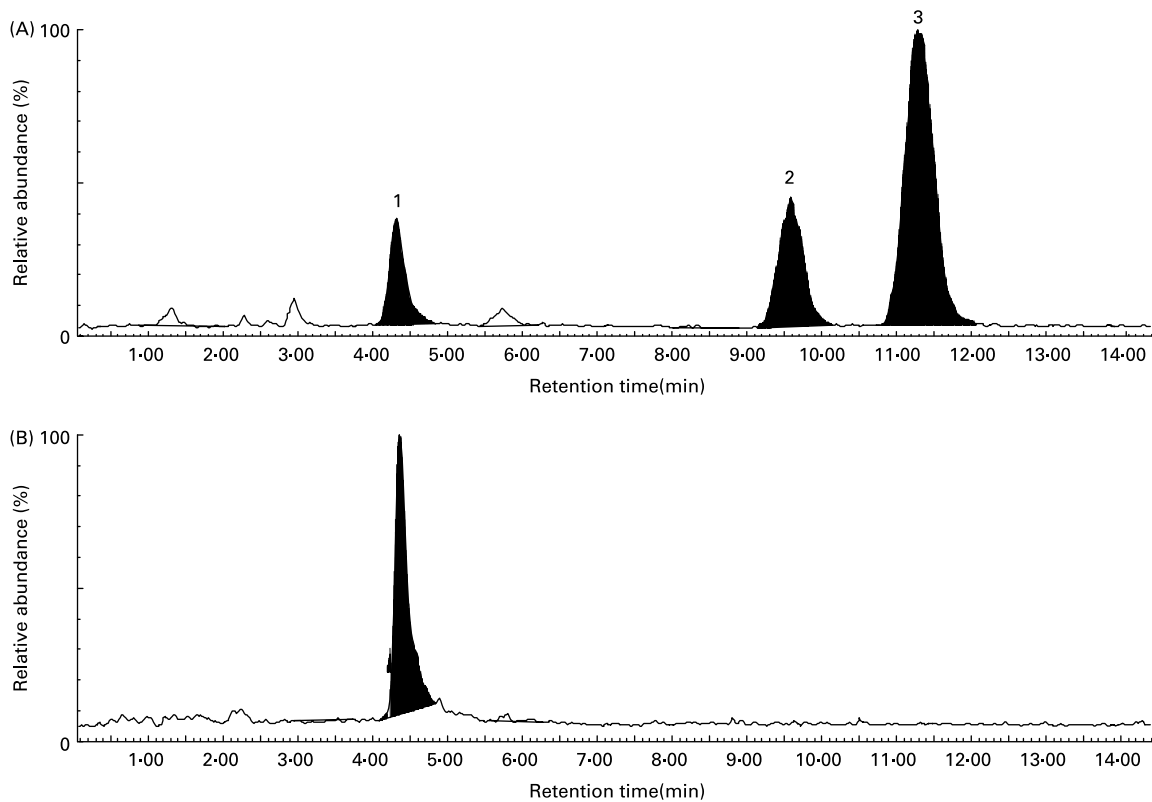
Fig. 2. Typical liquid chromatography (LC)−MS/MS chromatograms (multiple reaction monitoring mode) of a plasma sample (subject 3) collected 5 h after the intake of 300 ml blood orange juice (A) and before intake (B). Peak 1 represents the internal standard (morin) (transition $(m/z)^- \ 301 \rightarrow 151$). Peak 2 represents naringenin (transition $(m/z)^- \ 271 \rightarrow 151$). Peak 3 represents hesperetin (transition $(m/z)^- \ 301 \rightarrow 164$, $(m/z)^- \ 301 \rightarrow 151$).

In plasma, the electrospray ionisation negative collisionally activated dissociation MS (CAD-MS/MS) of naringenin gave a product ion with $(m/z)^- \ 151$. The $(m/z)^- \ 151$ is a common ion product for flavanones and is believed to result from the retro-Diels–Alder reaction of the flavonoid A ring. In the applied conditions, hesperetin also gave the product ion with $(m/z)^- \ 151$ but the most abundant ion product was the ion with $(m/z)^- \ 164$.

Naringenin and hesperetin plasma concentrations were determined in multiple reaction monitoring mode using the transitions $(m/z)^- \ 271 \rightarrow 151$ and $(m/z)^- \ 301 \rightarrow 164$, $(m/z)^- \ 301 \rightarrow 151$, respectively. For morin (I.S.) we used the transition $(m/z)^- \ 301 \rightarrow 151$.

The results show that naringenin and hesperetin were absorbed from BOJ by all the subjects. However, relevant inter-individual variations were observed as indicated by the

Table 1. Concentration of flavanones in plasma (ng/ml) before ($t = 0$) and after drinking 150 or 300 ml blood orange juice (BOJ)

(Mean values and standard deviations)

	Hesperetin				Naringenin			
	300 ml		150 ml		300 ml		150 ml	
t (h)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
1	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
2	4·4	7·6	1·4	3·8	1·2	2·0	1·2	3·1
3	13·4	16·6	12·8	26·2	7·6	7·0	3·7	8·0
4	36·0	38·6	23·9	37·3	16·8	17·0	10·4	14·6
5	73·2	62·2	33·0	31·6	34·0	20·6	13·4	6·8
6	55·7	37·6	33·2	27·7	13·6	9·7	7·4	4·5
7	35·9	34·5	20·5	25·7	5·5	4·5	4·1	3·9
8	24·7	40·7	8·4	11·2	2·2	2·9	1·7	2·6
24	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
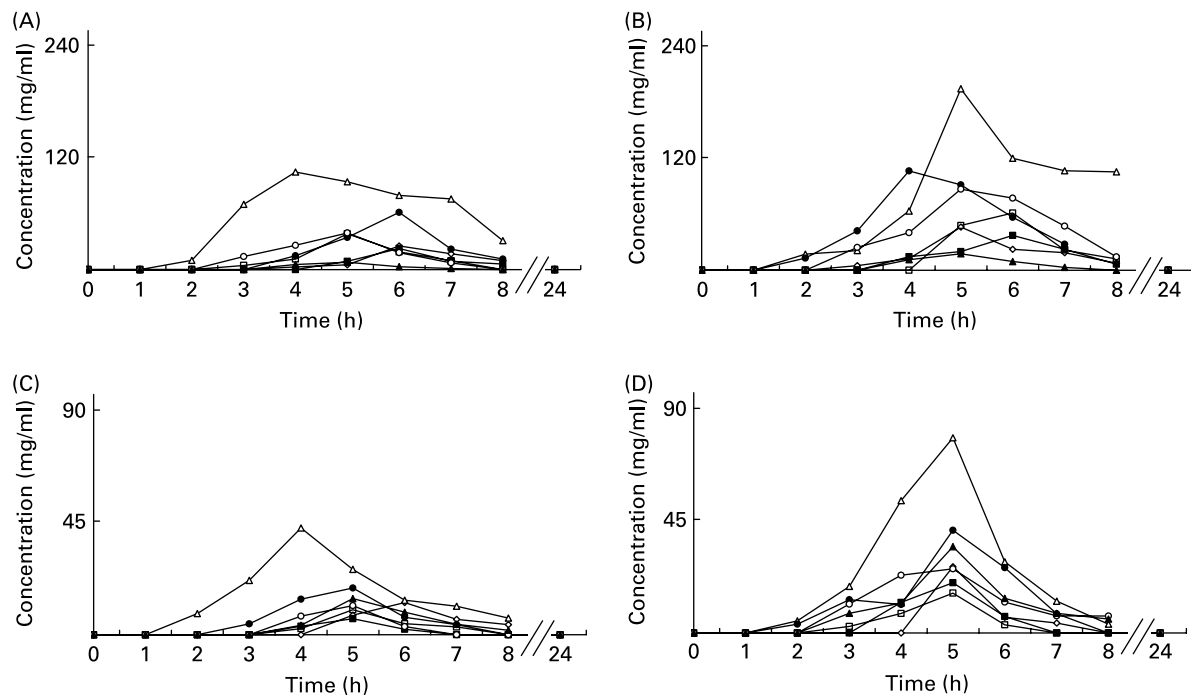
Fig. 3. Individual plasma concentration curves for hesperetin (A, B) and naringenin (C, D) after the consumption of 150 ml (A, C) and 300 ml (B, D) of blood orange juice. (◇), Subject 1; (■), subject 2; (△), subject 3; (●), subject 4; (□), subject 5; (○), subject 6; (▲), subject 7.

high standard deviations in the C_{max} and AUC values (Table 2) in subjects receiving the same BOJ dose. The subjects were all female students with comparable age, weight and food habits. For these reasons, it seems that the inter-individual variability was not dependent on these parameters. As hypothesised by Erlund *et al.* (2001) these variations could be due to different gastrointestinal microflora composition. In fact, the intestinal human microflora would not be able to remove the sugar moiety or it may metabolise the aglycones into phenolic acids (Felgines *et al.* 2000). Recently, Nielsen *et al.* (2006) demonstrated that the bioavailability of hesperidin was modulated by the enzymic conversion to hesperetin-7-glucoside, changing the absorption site from the colon to the small intestine.

Table 2. Plasma kinetic indexes in healthy volunteers (*n* 7) for hesperetin and naringenin after ingestion of 150 or 300 ml of blood orange juice (Individual values, group mean values and standard deviations)

Subject	t_{max} (h)		C_{max} (ng/ml)		AUC (ng × h)/ml		AUC/dose (ng × h)/ml	
	150 ml	300 ml	150 ml	300 ml	150 ml	300 ml	150 ml	300 ml
Hesperetin								
1	6	5	25·6	46·9	133·3	170·8	2·6	1·7
2	6	6	22·9	37·9	93·6	151·2	1·8	1·5
3	4	5	106·0	197·5	702·9	1433·1	13·7	14·0
4	6	4	62·3	108·1	231·1	336·1	4·5	3·3
5	5	6	33·8	62·2	83·6	237·4	1·6	2·3
6	5	5	39·9	88·2	106·0	404·4	2·1	3·9
7	5	5	7·8	17·9	17·8	41·8	0·3	0·4
Mean	5·3	5·1	43·4	79·8	195·5	396·4	3·8	3·8
SD	0·8	0·7	32·4	60·1	232·7	472·6	4·5	4·6
Naringenin								
1	6	5	12·6	26·0	60·1	36·1	10·0	3·0
2	5	5	6·3	19·8	10·0	38·2	1·7	3·2
3	4	5	42·0	76·8	173·9	219·5	29·0	18·3
4	5	5	18·4	40·4	45·4	99·7	7·6	8·3
5	5	5	9·8	15·7	19·6	29·1	3·3	2·4
6	5	5	11·5	25·4	21·9	129·3	3·7	10·8
7	5	5	14·2	33·9	46·0	117·5	7·7	9·8
Mean	5·0	5·0	16·4	34·0	53·9	95·6	9·0	8·0
SD	0·6	0·0	11·9	20·6	55·8	68·6	9·3	5·7

t_{max}, Time taken to reach peak concentration; C_{max}, peak concentration; AUC, area under the curve.

After the BOJ ingestion, naringenin and hesperetin plasma concentrations increased, reaching a peak at about 5·0 h, indicating that these molecules are absorbed from the distal parts of the small intestine or the colon. After 24 h the flavanone plasma levels returned to basal values. However, in several subjects, hesperetin and naringenin conjugates soon appeared in plasma (2–3 h) after consumption of BOJ containing their glycosides, indicating a possible absorption from the proximal part of the small intestine. Some authors have reported flavonoid absorption from the small intestine (Hollman *et al.* 1999; Walgren *et al.* 2000) and small-intestinal enzymes able to hydrolyse flavonoid glycosides have also been isolated (Day *et al.* 1998, 2000). On the other hand, the isolated enzymes were able to hydrolyse several flavonoid glucosides but not naringin. Neither hesperidin, nor its hydrolysis or glucuronidation products, was detected in the lumen after the perfusion of the jejunum and ileum with hesperidin (Spencer *et al.* 1999). Moreover, after a single oral administration of naringin, intact naringin was isolated after 2–4 h in human urine (Ishii *et al.* 2000). This study suggests that a small amount of naringin can be rapidly absorbed from the human gastrointestinal tract. Other studies, involving the absorption of anthocyanins, demonstrated that these flavonoids were rapidly and efficiently absorbed from the stomach (Passamonti *et al.* 2003; Talavera *et al.* 2003).

The present results suggest that flavonoid uptake occurs not only in the large intestine, where bacterial enzymes hydrolyse glycosides before absorption of the aglycone, but also in the small intestine; where small amounts of flavonoids can be absorbed.

Hesperetin and naringenin maximum plasma concentrations, after the ingestion of 300 ml BOJ, were in the range 15–200 and 15–80 ng/ml, respectively. The results are comparable with those of a study (Manach *et al.* 2003) carried out using different blond orange juice quantities administered with a meal. Moreover, in an another study (Erlund *et al.* 2001), the authors reported similar kinetic curves but higher plasma hesperetin concentrations ranging from 150 to 1500 ng/ml after the ingestion of blond orange juice providing about 125 mg hesperetin. On the other hand, other studies on flavonoid bioavailability in human subjects report plasma concentrations comparable with those found in the present study (Manach *et al.* 2005*b*).

In BOJ, the hesperidin concentration was about eight times that of narirutin, so it is not surprising that the naringenin concentrations found in plasma were lower than those of hesperetin. However, for naringenin, the $AUC_{0-24 h}$/mg ingested flavanones ratio was higher than for hesperetin as previously observed by Franke *et al.* (2005). This seems to indicate that naringenin was more bioavailable than hesperetin.

In conclusion, the present study describes the pharmacokinetics of hesperetin and naringenin in human subjects and provides evidence that the concentrations in plasma rapidly return to their basal values. For this reason it is important to have a habitual intake of BOJ to have a possible health benefit.

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