Plasma pharmacokinetics of (poly)phenol metabolites and catabolites after ingestion of orange juice by endurance trained men

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Running heading: Bioavailability of flavanones metabolites and catabolites

Highlights

- Human absorption and metabolism of orange juice (poly)phenols

- Mammalian and microbiota-mediated conversions

- Plasma pharmacokinetic of flavanone metabolites and catabolites

- Unexpected behaviour of 3-hydroxy-3-(phenyl)propanoic acids highlighted

- Essential data for ex vivo cell studies on the protective effects of flavanones

Abstract

The health benefits of orange juice (OJ) consumption are attributed in part to the circulating flavanone phase II metabolites and their microbial-derived ring fission phenolic catabolites. The present study investigated these compounds in the bloodstream after acute intake of 500 mL of OJ. Plasma samples obtained at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after OJ intake were analysed by HPLC-HR-MS. Eleven flavanone metabolites and 36 phenolic catabolites were identified and quantified in plasma. The main metabolites were hesperetin-3´-sulfate with a peak plasma concentration (C_{max}) of 80 nmol/L, followed by hesperetin-7-glucuronide (C_{max} 24 nmol/L), hesperetin-3´-glucuronide (C_{max} 18 nmol/L) and naringenin-7-glucuronide (C_{max} 21 nmol/L). Among the main phenolic catabolites to increase in plasma after OJ consumption were 3´-methoxycinnamic acid-4´-sulfate (C_{max} 19 nmol/L), 3-hydroxy-3-(3'-hydroxy-4'-methoxyphenyl)propanoic acid (C_{max} 20 nmol/L), 3-(3'-hydroxy-4'-methoxyphenyl)propanoic acid (C_{max} 19 nmol/L), 3-(4'-hydroxyphenyl)propanoic acid (C_{max} 25 nmol/L), and 3-(phenyl)propanoic acid (C_{max} 19 nmol/L), as well as substantial amounts of phenylacetic and hippuric acids. The comprehensive plasma pharmacokinetic profiles that were obtained are of value to the design of future ex vivo cell studies, aimed at elucidating the mechanisms underlying the potential health benefits of OJ consumption.

1. Introduction

Orange juice (OJ) flavanones, mainly hesperetin-7-*O*-rutinoside (hesperidin) and naringenin-7-rutinoside (narirutin), have been reported to have a wide range of effects on human health [1,2]. Epidemiological and clinical studies suggest that a higher intake of citrus flavanones is associated with a 19% decreased risk of stroke [3], and significant improvements in HDL-cholesterol, triacylglycerol, and postprandial microvascular endothelial reactivity, low-density lipoprotein-cholesterol, glucose and insulin sensitivity [4]. Furthermore, flavanone intake has been inversely correlated with postprandial lipid response to a suppressed fatty acid synthesis in the liver, with a consequent reduction in triglyceride production and VLDL secretion in subjects with high cardiovascular risk [5]. A randomised, 12 week, double-blind crossover study has shown that the consumption of OJs containing either normal or a high concentration of flavanones protected against DNA damage and lipid peroxidation, modified the activity of antioxidant enzymes, and reduced body weight in overweight or obese non-smoking adults [6]. Other intervention studies have found that regular consumption of OJ inhibits oxidative stress, inflammatory responses [7] and brings about an improvement in vascular function [8].

The health benefits of OJ consumption are attributed to the absorbable and circulating flavanone metabolites and gut microbial catabolites. Early reports showed that following ingestion of OJ, the sugar moiety of flavanone-*O*-rutinosides is cleaved in the distal gastrointestinal (GI) tract and the released aglycones are absorbed appearing in the bloodstream as glucuronide and sulfate metabolites. Quantification of metabolites in plasma and urine was based on the amounts of aglycone released by glucuronidase/sulfatase treatment of extracts prior to analysis by HPLC and as a consequence provides only limited information on the identity of the metabolites [9–11].

The advent of HPLC-MS, and the availability of metabolites as reference compounds, facilitated the analysis of samples without recourse to enzyme hydrolysis. Brett et al. [12] were the first to identify flavanone metabolites reporting the presence of the 3′-

and 7-glucuronides of hesperetin and the 4'- and 7-glucuronides of naringenin in both plasma and urine after OJ intake. Bredsdorff et al. [13] also identified these four glucuronides in urine, along with hesperetin-3'-sulfate, hesperetin-3',7-diglucuronide and hesperetin-5,7-diglucuronide, after ingestion of an α-rhamnosidase-treated OJ which contained hesperetin 7-*O*-glucoside rather than the 7-*O*-rutinoside.

Traditionally, the bioavailability of OJ flavanones was considered to be low with only relatively small amounts of metabolites entering the systemic circulation [12,14,15]. However, when phenolic catabolites produced by microbiota-mediated ring fission of hesperetin and naringenin were included in estimates of urinary excretion, the overall bioavailability of the flavanones was recognized as being much greater than previously envisaged [1,16].

The volunteers used in the current bioavailability study with OJ were endurance trained men. An earlier publication provided information on urinary excretion of flavanones metabolites and catabolites after OJ consumption and the effect of cessation of training for a period of 7-days [17]. This paper provides comprehensive information on the pharmacokinetics of hesperetin and naringenin metabolites in the blood stream after the consumption of OJ, and also on the appearance of microbiota-derived catabolites including cinnamic, 3-(phenyl)propanoic, phenylacetic and hippuric acids. The information is of value as it will assist the design of future ex vivo cell studies aimed at elucidating the mechanisms underlying the potential health benefits of OJ consumption.

2. Materials and methods

2.1. Chemicals

4-Hydroxybenzoic acid, 3-hydroxybenzoic acid, 4´-hydroxy-3´-methoxycinnamic acid, 3´-hydroxy-4´-methocycinnamic acid, 3´,4´-dihydroxycinnamic acid, 4'-hydroxyphenylacetic acid, 3'-hydroxyphenylacetic acid, 3',4'-dimethoxyphenylacetic acid, 4'-hydroxy-3'-methoxyphenylacetic acid, hippuric acid, 3,4-dihydroxybenzoic acid,

4-hydroxy-3-methoxybenzoic acid, 3-hydroxy-4-methoxybenzoic acid, 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid, 3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid, 2-hydroxy-2-(4′-hydroxy-3´-methoxyphenyl)acetic acid, 3-(3′,4′-dihydroxyphenyl)propanoic acid, 3-(3′,4′-dihydroxyphenyl)acetic acid, 3-(4′-hydroxyphenyl)propanoic acid, phenylacetic acid, 3-(phenyl)propanoic acid, 1,3,5-trihydroxyphenol, 3′-hydroxycinnamic acid, 4′-hydroxycinnamic acid and *p*-sympatol were obtained from Sigma-Aldrich (Poole, Dorset, U.K.). Hesperetin-7-glucuronide, naringenin-4′-glucuronide, and naringenin-7-glucuronide were purchased from Toronto Research Chemicals (Toronto, Canada). Hesperetin-3´-sulfate was a generous gift from Dr. Christine Morand (INRA/Clemont-Ferrand, France). 3′-hydroxyhippuric acid and 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid were purchased from Toronto Research Chemicals (Toronto, Canada). Cinnamic acid-3′-glucuronide, cinnamic acid-4′-glucuronide, 4′-hydroxycinnamic acid-3′-glucuronide, 3′-hydroxycinnamic acid-4′-glucuronide, 4′-hydroxycinnamic acid-3′-sulfate, 3′-methoxycinnamic acid-4′-glucuronide, 3′-methoxycinnamic acid-4′-sulfate, 4′-methoxycinnamic acid-3′-glucuronide, 3-(3′-hydroxyphenyl)propanoic acid-4′-glucuronide, 3-(phenyl)propanoic acid-4′-glucuronide, 3-(4′-hydroxyphenyl)propanoic acid-3′-glucuronide, 3-(3′-hydroxyphenyl)propanoic acid-4′-sulfate, 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate, 3-(3′-methoxyphenyl)propanoic acid-4′-glucuronide, 3-(4′-methoxyphenyl)propanoic acid-3′-glucuronide, and 3-(3′-methoxyphenyl)propanoic acid-4′-sulfate were kindly provided by Denis Barron (Nestle Research Center, Lausanne, Switzerland) and Gary Williamson (Monash University, Notting Hill, Victoria, Australia). 4′-Hydroxyhippuric acid was obtained from Bachem (UK) Ltd. (St. Helens, UK). 3-(3′-Hydroxyphenyl)propanoic acid was supplied by Fluorochem (Derby, UK). 3-Hydroxy-3-(3´-hydroxy-4′-methoxyphenyl)propanoic acid was isolated in a previous study [16]. Formic acid and HPLC-MS-grade methanol were obtained from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Note, the names for phenolic catabolites and their phase II metabolites used in this paper is based on the nomenclature thesaurus of Kay et al. [18] and the nomenclature used in cited papers has, as necessary, been adjusted.

2.2. Participants

Thirteen endurance trained, Caucassian men (10 distance runners, 3 cyclists) aged 31.8 ± 5.7 years, with a body mass index of 21.7 ± 1.8 kg/m^2, percentage body fat of $7.5 \pm 2.9\%$ and maximal oxygen consumption of 58.2 ± 5.3 mL/kg/min (all mean values $\pm$ SD) volunteered to participate in the study. The participants had been training regularly for the past 4-12 years and typically performed 5-10 h of endurance training per week. They competed regularly in running events, such as marathons and half-marathons, at regional and national levels. All were apparently healthy normotensive non-smokers and were not taking any drug therapies or supplements. Exclusion criteria included: history of gastrointestinal disease, eating disorders and being vegetarian. Written informed consent was provided by all participants. Procedures for screening fitness were as described previously by Pereira-Caro et al. [17].

Of the 12 participants who completed the trial, two were excluded, as they did not follow a pre-feed low (poly)phenol diet. The study was approved by the College of Medical, Veterinary and Life Sciences Ethics Committee of the University of Glasgow and registered at ClinicalTrials.gov (NCT02627547).

2.3. Study design

Each subject participated in a 24 h OJ feeding trial during a period of normal training. Participants weighed and recorded their dietary intake and were asked to follow a diet low in (poly)phenolic compounds by avoiding fruits and vegetables, nuts, high-fibre products, and beverages such as tea, coffee and fruit juices, as well as to abstain from consuming alcohol, for the 2 days prior to the OJ feeding trial. Homogeneity

of the ingested OJ (Tropicana "With Bits", purchased from a local supermarket) was ensured by mixing in bulk and storing 500 mL aliquots at −80°C.

On the morning of the feeding trials, participants reported to the metabolic laboratory between 0800 h and 0900 h after a 12-h fast and brought their excreted overnight urine sample. Height, body mass and body fat were measured. A venous cannula was inserted and after a 10 min interval a 7 mL baseline blood sample was obtained. Participants then consumed 500 mL of OJ and, except for water intake to maintain adequate levels of hydration, no other food or drink was consumed for the next 4 h. Further blood samples were obtained 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after OJ intake. Four hours after collection of the first blood sample participants were provided with a white roll with butter. After blood collection at 8 h, the cannula was removed, and participants were provided with a standard low (poly)phenol meal (a buttered white roll with ham and cheese, and crisps) after which they left the laboratory to sleep at home. They were instructed to continue the low (poly)phenol diet that evening and return to the laboratory the next morning in the fasted state, with their overnight urine, to give the 24 h blood sample. During the feeding trial participants collected all urine excreted over the following time periods: 0-2, 2-5, 5-8, 8-10 and 10-24 h. Analysis of these samples was reported in an earlier publication [17].

Blood samples were collected in potassium EDTA tubes (BD Vacutainer Systems, UK) and placed immediately on ice. Within 15 min of collection blood was centrifuged at 2000 g for 15 min at 4°C and plasma was stored at −80 °C prior to analysis.

2.4. Extraction and analysis of orange juice

The pulp-enriched OJ used in the feeding study was extracted using the following procedure. Briefly, 5 mL aliquots of juice, previously homogenised using an Ultraturrax homogenizer, were extracted twice with 5 mL of methanol for 2 min and centrifuged at 2800 g for 15 min at 4 °C. The pellet was extracted in the same manner with 2 mL of methanol. The two supernatants were pooled and reduced to dryness in vacuo,

redissolved in 6 mL of 50 % aqueous methanol and stored at –80 °C prior to analysis as described previously by Pereira-Caro et al. [17].

2.5. Processing of plasma

Plasma samples were defrosted, vortexed and 400 μL aliquots were mixed with 1 mL of 2% formic acid in acetonitrile. The samples were vortexed and ultrasonicated for 10 min. After centrifugation at 16100 g for 10 min, supernatants were reduced to dryness in vacuo using a Speedvac concentrator (Thermo Fisher Scientific Inc., San Jose, CA) and resuspended in 100 μL of methanol:water:formic acid (50:50:0.1, v/v/v) which was centrifuged at 16100 g for 10 min and 5 μL aliquots of the supernatant analysed by HPLC-HR-MS (high performance liquid chromatography–high resolution-mass spectrometry).

2.7. Analysis of plasma

Extracted plasma samples were analysed by HPLC-HR-MS using a Dionex Ultimate 3000 RS UHPLC system coupled to Exactive Orbitrap mass spectrometer (ThermoFisher Scientific, San Jose, CA). The analysis protocol has been previously described and validated to detect and quantify 46 analytes relative to commercial and synthetic reference standards [19]. Briefly, reverse phase separation was carried out using a 150 x 4.6 mm i.d. 5 μm 100A C18 Kinetex column (Phenomenex, Macclesfield, UK) maintained at 40 °C and eluted at a flow rate of 1.0mL/min with a 45 min gradient of 3-50% of 0.1% acidic methanol in 0.1% aqueous formic acid. After passing through the flow cell of the PDA detector the column eluate was split and 0.2 mL/min directed to an Exactive Orbitrap mass spectrometer fitted with a heated electrospray ionization probe operating in negative ionization mode. Data acquisition and processing were carried out using Xcalibur 3.0 software.

Identification of flavanone metabolites and phenolic and aromatic acid catabolites in plasma was performed by comparing the exact mass and the retention time with

available standards. Metabolites without reference standards were tentatively identified by comparing the theoretical exact mass with the measured accurate mass of the molecular ion and the accompanying fragmentation pattern. The identifications were categorized according to the Metabolite Standards Initiative Metabolomics Identification (MSIMI) levels [20]. Quantification of metabolites was carried out by selecting the theoretical exact mass of the molecular ion by reference to standard curves. In absence of reference compounds, metabolites were quantified by reference to the calibration curve of a closely related parent compound as described by Ordóñez et al. [19].

2.8 Statistical analysis

Statistical analyses were performed on the basis of three analytical replicates measured on each sample. A one-way ANOVA was carried out to assess for significant differences (level of significance p-value <0.05) using R software (v3.5.0). Fisher's LSD pairwise comparison was performed on the data.

3. Results

3.1. Identification and quantification of (poly)phenols in OJ

The 500 mL OJ consumed by the volunteers contained hesperetin-7-rutinoside (246 µmol), hesperetin-7-rutinoside-3′-glucoside (4 µmol), naringenin-7-rutinoside (62 µmol), 4′-methoxy-naringenin-7-rutinoside (14 µmol), eriodictyol-7-rutinoside (4 µmol), apigenin-6,8-*C*-diglucoside (35 µmol), 3′-methoxycinnamic acid-4′-*O*-glucoside (16 µmol), cinnamic acid- 4′-glucoside (11 µmol), a sinapic acid-hexoside (6 µmol), and amine *p*-sympatol (aka *p*-synephrine) (6 µmol) (for structures see [21] and Fig. S1 in Supplementary Information). In total, the ingested juice contained 398 µmol (poly)phenols, of which 330 µmol were flavanones. The basis of the identification and quantification of these compounds was described previously by Pereira-Caro et al. [21].

3.2.   Plasma levels of hesperetin and naringenin metabolites

Following acute ingestion of the OJ supplement, eight hesperetin phase II sulfate and glucuronide metabolites (see [21] and Fig. S2 for structures) were detected in plasma while there was an absence of the parent rutinoside and flavanone aglycones. The plasma profiles are illustrated in Fig. 1 with pharmacokinetic analyses in Table 1. Hesperetin-3′-sulfate was the dominant metabolite with a maximum concentration (C_{max}) of 80 nmol/L and a 0-8 h area-under-the-curve ($AUC_{0-8\,h}$) of 280 nmol/h/L. A sulfate, C_{max} 3.3 nmol/L, previously tentatively identified as hesperetin-5- or 7-sulfate [21,22], and a sulfoglucuronide (11 nmol/L) were comparatively minor components. Two glucuronides were detected, hesperetin-7-glucuronide (24 nmol/L) and hesperetin-3′-glucuronide (18 nmol/L) along with 1.3-3.2 nmol/L concentrations of three hesperetin diglucuronides (Fig. 1, Table 1). Three naringenin metabolites were detected in plasma, namely naringenin-7-glucuronide (C_{max} 21 nmol/L), naringenin-4′-glucuronide (14 nmol/L) and narigenin-4′-sulfate (11 nmol/L) (Fig. 2, Table 1). Probably a consequence of the relatively low intake of eriodictyol-7-*O*-rutinoside, no eriodictyol metabolites were detected in plasma although as reported previously 0.2 nmol/L of an eriodictyol sulfate and a sulfo-glucuronide, equivalent to 5.0% of intake, were excreted in urine (Table S1) [17].

The main hesperetin and naringenin metabolites appeared in the plasma of all 10 volunteers while those in lower concentrations were present in detectable quantities of 6-10 subjects. None of the metabolites were detected in 0 h plasma collected immediately prior to OJ consumption (Table 1).

3.3.   Plasma levels of phenolic catabolites

Of the 36 phenolic catabolites detected in plasma after OJ consumption, 20 were in sufficient quantities to provided discernable pharmacokinetic profiles (Table 2, Figs. 3-6). Among the phenolic acids that increased was 4′-hydroxy-3′-methoxycinnamic acid (aka ferulic acid) which had a relatively low 1.5 nmol/L C_{max} 1.0 h after OJ ingestion

10

and declined thereafter to near baseline levels with a minor secondary peak at 6.0 h (Fig. 3, Table 2). The 4′-sulfate and 4′-glucuronide of 3′-methoxycinnamic acid were also detected at peak concentrations of 3.5 and 19 nmol/L, with respective T_{max} values at 1.7 and 1.2 h. Other compounds with a 3′-methoxy group indicating that they too were probably derived from 4′-hydroxy-3′-methoxycinnamic acid, were trace levels of 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid (aka dihydroferulic acid) together with its 4′-sulfate and 4′-glucuronide, with respective C_{max} concentrations of 4.0 and 4.7 nmol/L. However, the 6-7 h T_{max} times of the two-phase II metabolites (Fig. 3, Table 2) suggest that their absorption into the circulatory system occurred mainly in the lower rather than the upper GIT.

4′-Methoxy metabolites, probably originating from C-ring fission of hesperetin, released from its 7-*O*-rutinoside by hydrolysis in the lower bowel, include trace levels of 3′-hydroxy-4′-methoxycinnamic acid (aka isoferulic acid), along with 4′-methoxycinnamic acid-3′-glucuronide (C_{max} 1.3 nmol/L, aka isoferulic acid-3′-glucuronide), as well as more substantial amounts of 3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid (19 nmol/L, aka dihydro-isoferulic acid) and its 3′-glucuronide (6.7 nmol/L) and 3′-sulfate (9.2 nmol/L) (Fig. 4).

Among the main plasma catabolites was the hydracrylic acid 3-hydroxy-3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid (C_{max} 20 nmol/L, aka 3-(3′-hydroxy-4′-methoxyphenyl)hydracrylic acid). 3-Hydroxy-3-(3′-hydroxyphenyl)propanoic acid (aka 3-(3′-hydroxyphenyl)hydracrylic acid) unlike its 4′-methoxy derivative, had an unusually high plasma profile in that the average 0 h baseline level was 270 nmol/L which more than doubled to 628 nmol/L by four hours post-dosing and then declined progressively to the zero time level after 24 h (Table 2, Fig. 5).

Other 3-(phenyl)propanoid, phenylacetic, and hippuric acids with discernible pharmacokinetic profiles were also detected with 4′-hydroxyphenylacetic acid being a major catabolite (C_{max} 151 nmol/L) (Table 2, Fig.6). Some of these catabolites, which appeared in substantial amounts following OJ intake, were present in plasma at 0 h prior

to supplementation, as well as 24 h after intake. These included 3-(phenyl)propanoic acid, 3-(3′-hydroxyphenyl)propanoic acid, 3-(4′-hydroxyphenyl)propanoic acid, 3′-hydroxyphenylacetic acid, 4′-hydroxyphenylacetic acid, hippuric acid, 3′-hydroxyhippuric acid and 4′-hydroxyhippuric acid (Table 2, Fig. 7). Other phenolic acids detected in the plasma of most volunteers in trace amounts, increased intermittently following OJ intake, but without a clear pharmacokinetic profile, are listed in Table 2. The structures of the 35 phenolic catabolites and their phase II metabolites that were detected in plasma are illustrated in Fig. 7.

4. Discussion

4.1. Absorption of flavanone phase II metabolites

The 4.2-6.8 h plasma T_{max} times of the flavanone metabolites (Table 1) are indicative of microbiota-mediated cleavage of the glycoside moiety from both hesperetin- and naringenin-O-rutinoside occurring principally in the distal GIT. This is followed by colonocyte- and/or hepatocyte-mediated phase II metabolism of the released hesperetin and naringenin which appear in the circulatory system as a variety of glucuronide and sulfate conjugates. This is supported by the findings of Actis-Goretta et al. [23] who perfused hesperetin-7-O-rutinoside directly into a 20-cm section of the proximal jejunum of healthy volunteers, using a multilumen intestinal perfusion catheter, and noted minimal excretion of hesperetin metabolites.

A number of the naringenin and hesperetin metabolites began appearing in plasma 2 h after OJ intake (Figs. 1 and 2) indicating that some absorption occurred in upper GIT. This is in keeping with the data of Borges et al. [24] which, after consumption of a drink containing hesperetin-7-O-rutinoside by ileostomists, demonstrated a 70% reduction in urinary excretion of hesperetin-glucuronides compared to subjects with a functioning colon.

The main hesperetin metabolite in plasma was hesperetin-3′-sulfate (Table 1) while hesperetin-3'-glucuronide dominates in urine [17]. Likewise, in the case of naringenin, there were broadly similar levels of naringenin-4′-glucuronide, naringenin-7-glucuronide and naringenin-4′-sulfate in plasma (Table 1, Fig. 2) while urine contained 3.8 μmol of the two glucuronides compared to 0.1 μmol of the 4′-sulfate (Table S1) [17].

The total $AUC_{0-8\,h}$ value for hesperetin metabolites was 487 nmol/h/L compared to 171 nmol/h/L for the naringenin metabolites, a 2.8-fold difference (Table 1). Hesperetin glycosides were ingested in ∼4.0-fold higher amounts than naringenin glycosides. Arguably, this indicates that naringenin glycosides are the more bioavailable which is in keeping with the relative recovery of metabolites in urine (Table S1) [17] and the earlier reports by Brett et al. [12] and Mullen et al. [25]. In this context it is of note that the mean plasma T_{max} for naringenin metabolites was 4.9 ± 0.6 h which is significantly earlier than the 6.4 ± 0.4 h T_{max} of the hesperetin metabolites (Table 1) indicating that while absorption of both flavanones occurred principally in the lower bowel absorption of naringenin metabolites is seemingly the more rapid of the two.

4.2. Absorption of phenolic catabolites and their phase II metabolites

Previously it was shown that after ingestion of the OJ, the volunteers excreted hesperetin, naringenin and eriodictyol phase II metabolites in 0-24 h urine in quantities equivalent to 4.2% of intake [17]. A much greater amount, 202 μmol, corresponding to 51% of intake, was excreted as a diversity of phenolic and aromatic compounds (Table S2) most of which were derived from microbiota-mediated ring fission of hesperetin and naringenin in the distal GIT [17]. Ring fission of flavanones typically releases 3-(phenyl)propanoic acids with methoxy and/or hydroxy substituent groups at the 3′ and/or 4′-positions. In vitro bacterial and fecal incubations have shown these C_6-C_3 compounds can be subject to demethylation and dehydroxylation steps as well as undergo shortening of the side chain to yield C_6-C_2 phenylacetic acids [22,26]. Although these is an absence of definitive feeding studies using labelled substrates, in vivo it is

possible that once absorbed, phenolic acids undergo further side chain shortening, catalysed by mammalian enzymes, forming C_6-C_1 benzoic acids which in the liver undergo glycination forming hippuric acids [1]. Trace amounts of 3,4-dihydroxybenzoic acid, the sulfate conjugates of 3-hydroxy- and 4-hydroxybenzoic acid, and more substantial amounts of hippuric acid, 3-hydroxyhippuric acid and 4-hydroxyhippuric acid were observed in plasma (Fig. 6, Table 2). Thus, it appears that glycination is the major phase II pathway for the metabolism of 3,4-dihydroxybenzoic acid.

Pathways for the conversions involved in the breakdown of hesperetin and naringenin after OJ consumption have been proposed [1,22] and the plasma pharmacokinetic data presented in Figures 1-6 and Table 2 provide insights into the formation of glucuronide and sulfate metabolites that occur at various points in the catabolism of the phenolic compounds derived from ring fission of the flavanones.

The phenylhydracrylic acids, 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid and its 4′-methoxy derivative, have been less studied than most other C_6–C_3 catabolites. Because the 3′-hydroxy-4′-methoxyphenyl-substitution pattern is comparatively rare, the identification of hesperetin as the source of 3-hydroxy-3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid is eminently plausible [1,16,22]. Its comparatively late plasma T_{max} (Table 2) associates its formation with activity of the gut microbiota although it has never been detected as a product of in vitro gut microbiota incubations [26-28]. 3-Hydroxy-3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid has been observed in the 24-hour urine of 30 out of 37 volunteers in three studies in which OJ was consumed, but was not detected when these volunteers ingested a placebo [16,17,22. Collectively, this suggests that there must be a gut microbiota precursor that is subject to endogenous metabolism after absorption.

3-Hydroxy-3-(3′-hydroxyphenyl)propanoic acid is considered a normal urinary metabolite [16,17, 22, 29] but is absent from or below the limit of detection in urine from ileostomists [30]. It does, however, have a very different plasma pharmacokinetic profile to its 4′-methoxy derivative with a substantial amount present pre-OJ dosing and

24 h after OJ intake (Fig. 5, Table 2). The increase in plasma post-dosing is plausibly connected to gut microbiota transformation of the OJ (poly)phenols, while the high background level may be a consequence of human metabolism unrelated to (poly)phenol intake. Excretion of 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid is reported to be influenced by single nucleotide polymorphisms in the *ABCC2* gene encoding multidrug resistance-associated protein-2 in the kidney [31]. Further investigations are required to clarify its origins.

It was previously reported that 0-24 h urinary excretion of 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid after OJ intake by the trained volunteers was 0.2 ± 0.3 µmol which was not a significant increase from baseline [17] (see Table S2). This level of 0-24 h urinary excretion for a catabolite with an $AUC_{0\text{-}8\,h}$ of 3202 ± 93 nmol/h/L is very small, especially when compared with values for urine of 18 ± 5.8 µmol and an $AUC_{0\text{-}8\,h}$ value of 57 ± 7 nmol/h/L for 3-hydroxy-3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid (Table 2). This implies that there may be further metabolism of 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid prior to excretion. Potentially, there could be conversion to glucuronide and/or sulfate metabolites. In subsequent preliminary HPLC-HR-MS analysis of urine samples we detected peaks with the appropriate fragmentation pattern and negatively charged molecular ions at *m/z* 357.0816, that corresponds with the glucuronide, and *m/z* 261.0063 which equates with the sulfate. Neither of these peaks were detected in plasma.

Some of the phenolic acid pharmacokinetic traces have T_{max} values of <2 h suggesting absorption in the proximal rather than the distal GIT (Figs. 3 and 4, Table 2). These include 3′-methoxy derivatives that were most probably derived, not from flavanones, but from the 3′-methoxycinnamic acid-4′-*O*-glucoside in the ingested OJ. It would appear that cleavage of the glucose moiety occurs in the small intestine and the released 4′-hydroxy-3′-methoxycinnamic acid is absorbed with a portion undergoing either 4′-glucuronidation or 4′-sulfation in enterocytes en route to the circulatory system. Feeding studies with coffee containing caffeoylquinic acids indicate that

although cinnamic acids are absorbed in the small intestine some pass to the distal GIT where they are converted by the microbiota to phenylpropanoic acids [32]. This may explain the presence of 3′-methoxylated phenylpropanoic acids in plasma with a T_{max} >6 h after OJ intake (Table 2, Fig. 5). However, eriodictyol is another possible source following a catechol-O-methyltransferase catalysed methylation of its catabolites.

2-Hydroxy-2-(4′-hydroxyphenyl)acetic acid had T_{max} of 1.9 h indicative of absorption in the small intestine (Fig. 5, Table 2). However, in view of the findings of Hengstmann and Aulepp [33], it is probably not a flavanone metabolite but originates from p-sympatol via a three step pathway with p-octopamine and 2-hydroxy-2-(4′-hydroxyphenyl)acetaldehyde acting as intermediates [1,22].

Further investigations on OJ (poly)phenol bioavailability, in particular clarification of the complex microbiota-mediated degradation pathways and the accompanying phase II metabolism of the products will require feeding studies with equimolar amounts of individual components that are labelled with radio/stable isotopes.

4.3. Potential bioactivity of metabolites and catabolites

There are investigations on the bioactivities of OJ flavanones, based on experiments using ex vivo cell models that have reported anti-inflammatory [34]), anti-diabetic effects [35], as well as beneficial impacts on vascular function [36]. However, these studies are of limited relevance as they were carried out with flavanone aglycones and rutinosides which do not appear in the circulatory system. There are, however, studies that have been carried out with hesperetin-3′-glucuronide, hesperetin-7-glucuronide and hesperetin-3′-sulfate, and naringenin-7-glucuronide which, the current investigation shows, do enter the bloodstream after OJ intake (Table 1). The reported effects include antiatherogenic properties [37,38], inhibition of low density lipoproteins oxidation [39], reduced TNF-α-induced migration of human aortic endothelial cell [40] and improved myeloid angiogenetic cell function [41].

There are also a number of studies on the bioactivity of colon-derived phenolic catabolites that used ex vivo test systems [see 42-46] and the topic has been reviewed by Kay et al. [1] and Williamson et al. [2]. There is evidence that mixtures of catabolites, as would occur in the circulation, have additive and even synergistic effects [43, 47-50]. In this context, the data obtained in the present study on the plasma C_{max} concentrations, of not just hesperetin and naringenin metabolites (Figs. 1 and 2, Table 1), but also those of the colonic catabolites and their phase II metabolites (Figs. 3-6, Table 2), is of value as for the first time it will enable combinations of these compounds to be assayed in ex vivo test systems in proportions that represent the relative amounts that appear in the circulatory system after OJ consumption.

5. **Conclusions**

This paper sheds new light on the complex transformations that occur during the passage of flavanones and other (poly)phenols through the GI tract following ingestion of OJ by endurance trained human subjects. Information is presented on plasma pharmacokinetics of glucuronide and sulfate metabolites of hesperetin and naringenin and other (poly)phenols. Data was also obtained on 36 cinnamic, 3-(phenyl)propanoic, phenylacetic, benzoic acids, and hippuric acid catabolites derived principally from microbiota-mediated breakdown in the GI tract. This information is of value to the design of ex vivo cell-based studies aimed at elucidating the underlying modes of action of the protective effects of OJ (poly)phenols on health and well-being. Furthermore, it is important to acknowledge that the bioavailability of (poly)phenols can be expected to be amended by environmental modulators such as diet, stress, supplement intake and exercise [51,52]. Thus, future studies should consider the impact of these compounding factors on plasma levels and urinary excretion of metabolites and gut-derived phenolic catabolites.

Author's contributions

D.M. contributed to the design of the study, supervised the cardiorespiratory fitness assessments of the volunteers and the feeding study, and helped in the preparation of the manuscript and its revisions. A.L.G contributed to the design of the study, and the preparation of the manuscript. T.P. and H.A. recruited participants and conducted the assessments of cardiorespiratory fitness and the feeding studies. G.P.-C. and I.A.L. conducted the HPLC-high-resolution mass spectrometry analyses and contributed to the drafting of the manuscript and its revisions. J.M.M.-R. contributed to the analytical aspects of the study and the drafting of the manuscript. M.N.C. supported the HPLC-high resolution mass spectrometry analyses, and helped in the preparation of the manuscript and its revisions. A.C. assisted in the design of the study, supported the HPLC-high resolution mass spectrometry analyses, and drafted the manuscript and its revisions.

Sources of funding

Declaration of interests

None.

Appendix A. Supporting information

Supplementary data supporting this article can be found in the online version at …..

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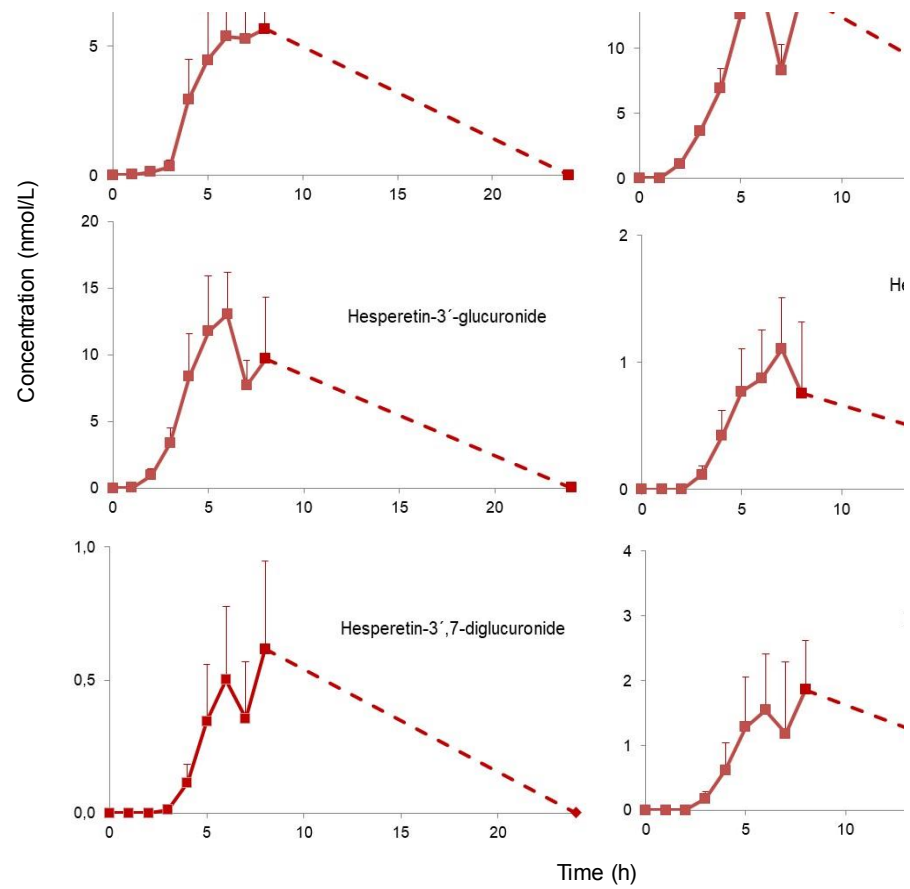
Fig. 1. Plasma pharmacokinetic profiles of hesperetin phase II metabolites 0-24 h after the ingestion of orange juice.
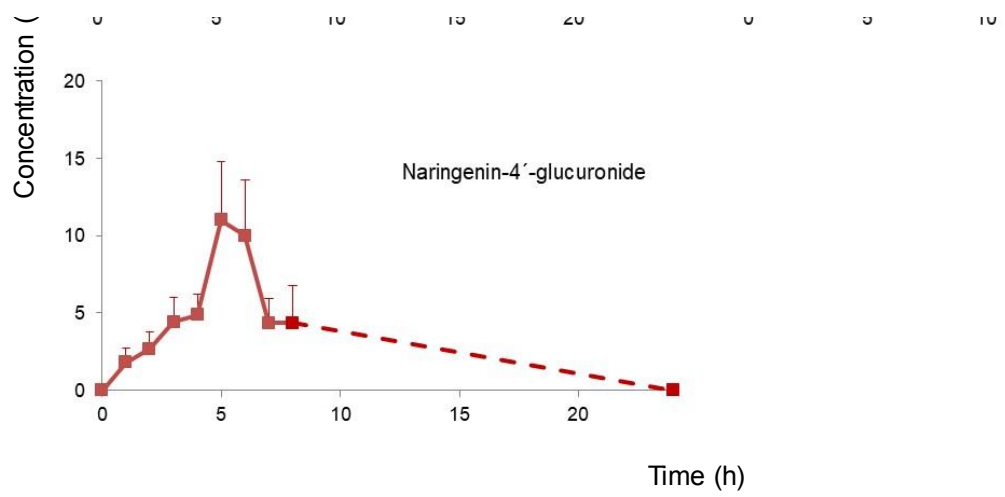
Fig. 2. Plasma pharmacokinetic profiles of naringenin phase II metabolites 0-24 h after the ingestion of orange juice.
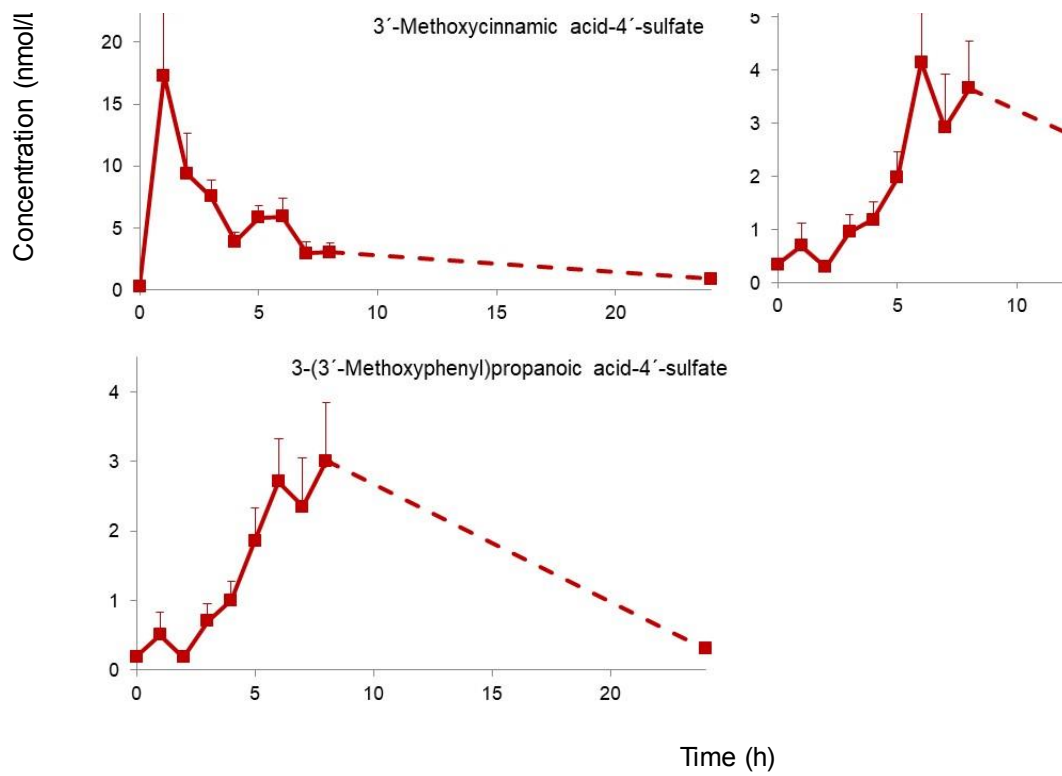
Fig. 3. Plasma pharmacokinetic profile of 3′-methoxyphenolic acids 0-24 h after the ingestion of orange juice.

3-(4´-Methoxyphenyl)propanoic acid-3´-glucuronide

2-Hydroxy-2-(4´-hydroxyphenyl)acetic acid

Time (h)

Concentration (nmol/L)
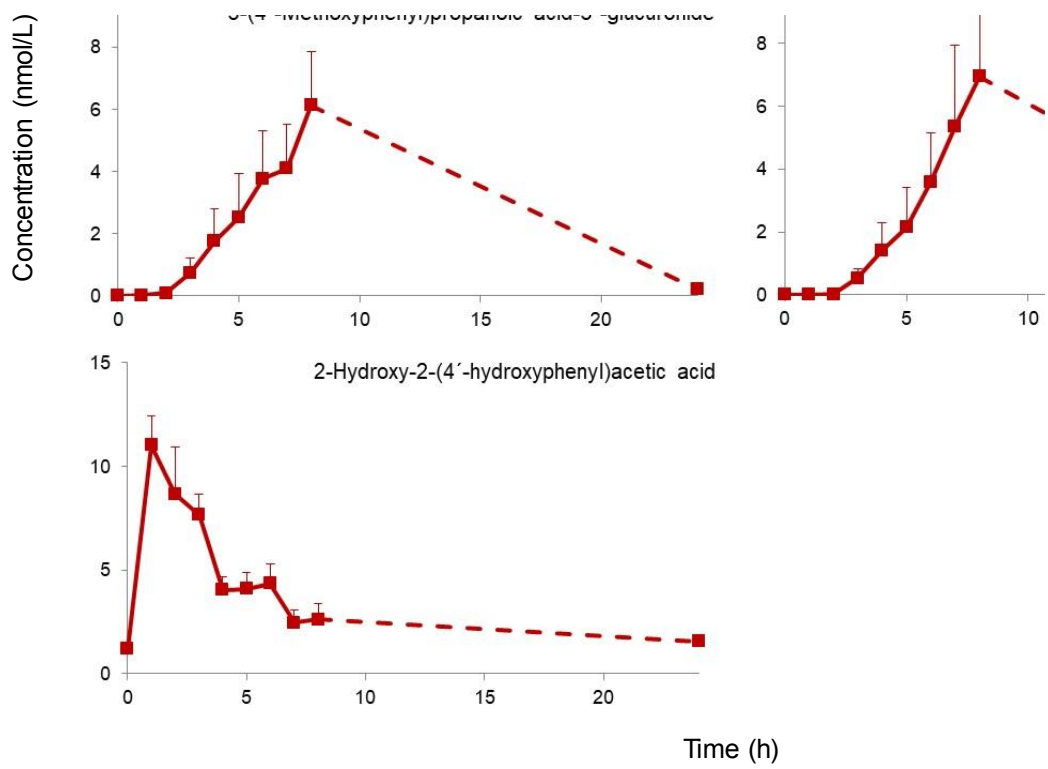
Fig. 4. Plasma pharmacokinetic profiles of 4′-methoxyphenolic acids and 2-hydroxy-2-(4′-hydroxyphenyl)acetic acid 0-24 h after the ingestion of orange juice.

Fig. 5. Plasma pharmacokinetic profiles of the phenylhydracrylic acids, 3-hydroxy-3-(3′-hydroxy-4′-methoxyphenyl)propanoic acid and 3-hydroxy-3-(3′-hydroxyphenyl)propanoic acid.
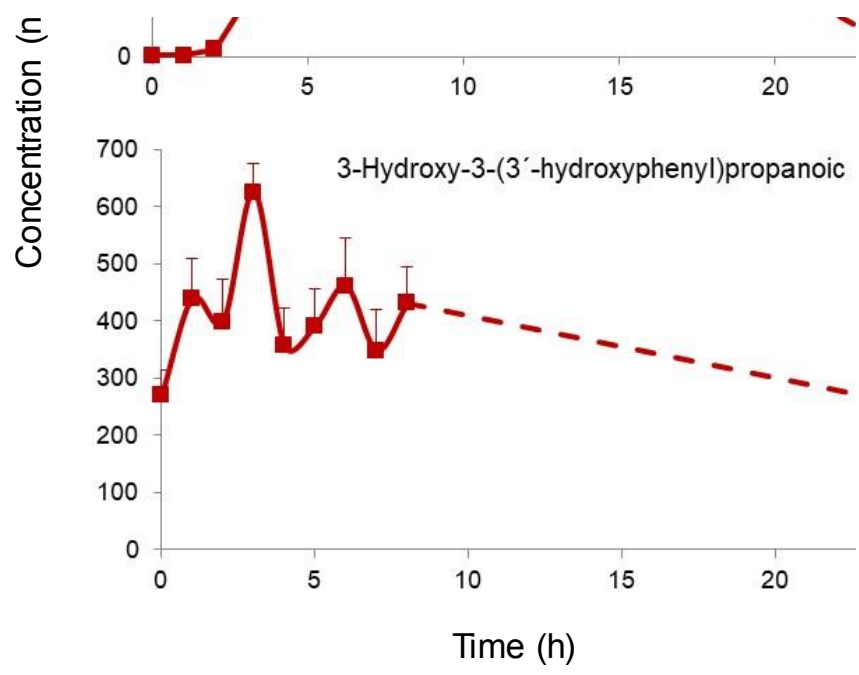
4´-Hydroxyphenylacetic acid

3´-Hydroxyhippuric acid
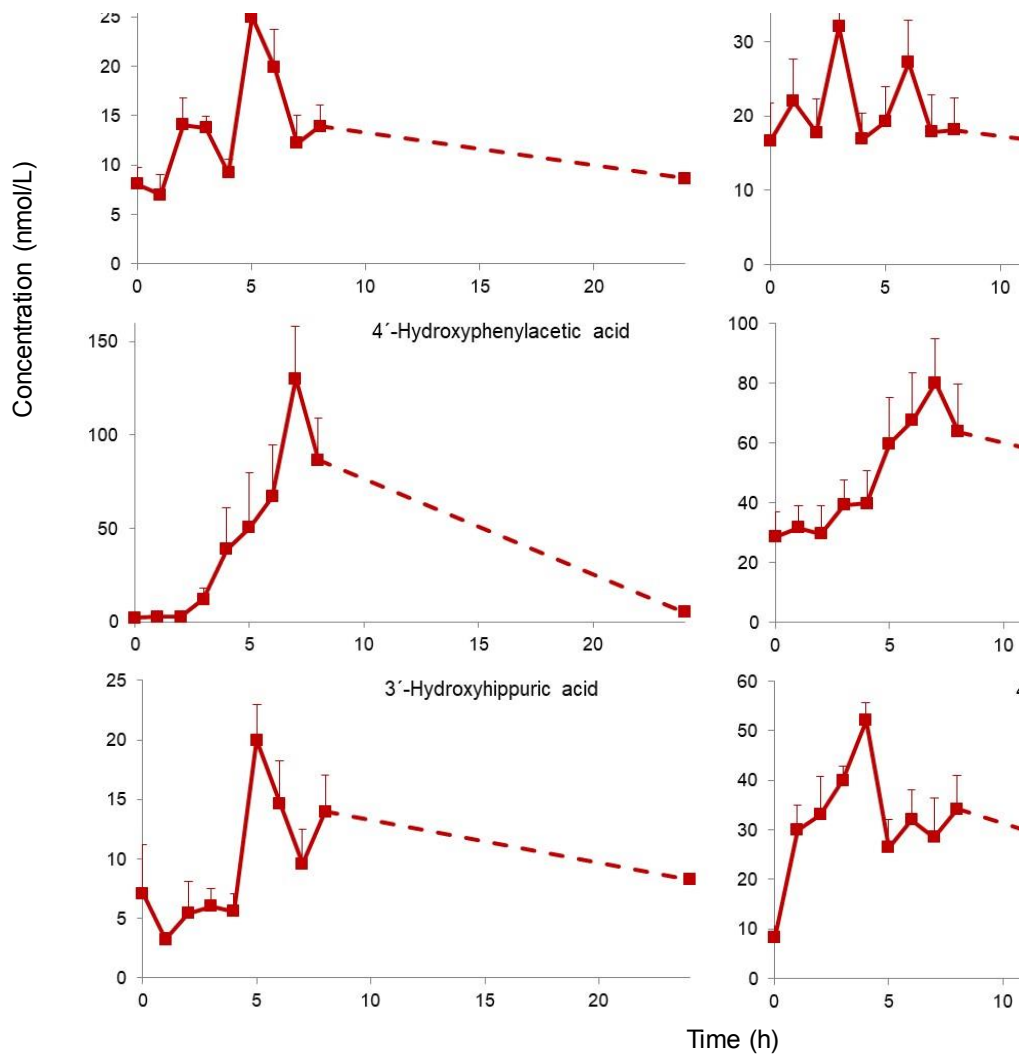
Fig. 6. Plasma pharmacokinetic profile of 3-(phenyl)propanoic, phenylacetic and hippuric acids 0-24 h after the ingestion of orange juice.

OCH₃ OSO₃⁻
HOOC
3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate

OH OCH₃
HOOC
3-(3'-Hydroxy-4'-methoxy phenyl)propanoic acid

OGlcUA OCH₃
HOOC
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide

HOOC
3-(4'-Methoxyphen acid-3'-sul

OH
HOOC
OH
3-Hydroxy-3-(3'-hydroxy phenyl)propanoic acid

OH OH
HOOC
3-(3',4'-Dihydroxyxphenyl) propanoic acid

OSO₃⁻ OH
HOOC
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate

HOOC
3-(3'-Hydroxyphe acid-4'-su

OH
HOOC
3-(4'-Hydroxyxphenyl) propanoic acid

HOOC
3-(Phenyl)propanoic acid

OH OSO₃⁻
HOOC
3'-Hydroxyphenylacetic acid-4'-sulfate

HOOC
3'-Methoxyphe 4'-su

OH OSO₃⁻
HOOC
3'-Hydroxyphenylacetic acid-4'-sulfate

OH
HOOC
3'-Hydroxyphenylacetic acid

OH
HOOC
4'-Hydroxyphenylacetic acid

HOOC
OH
2-Hydroxy-2-(4 3'-methoxy phen

OH OH
COOH
3,4,Dihydroxybenzoic acid

OSO₃⁻
COOH
Benzoic acid-3-sulfate

OSO₃⁻
COOH
Benzoic acid-4-sulfate

HOOC
N
H
O
Hippuric acid

HOOC
3'-Hyd

Fig. 7. Structures of phenolic acid catabolites and their phase II metabolites detected in plasma after consumption of orange juice. GlcUA - glucuronide

34

Table 1

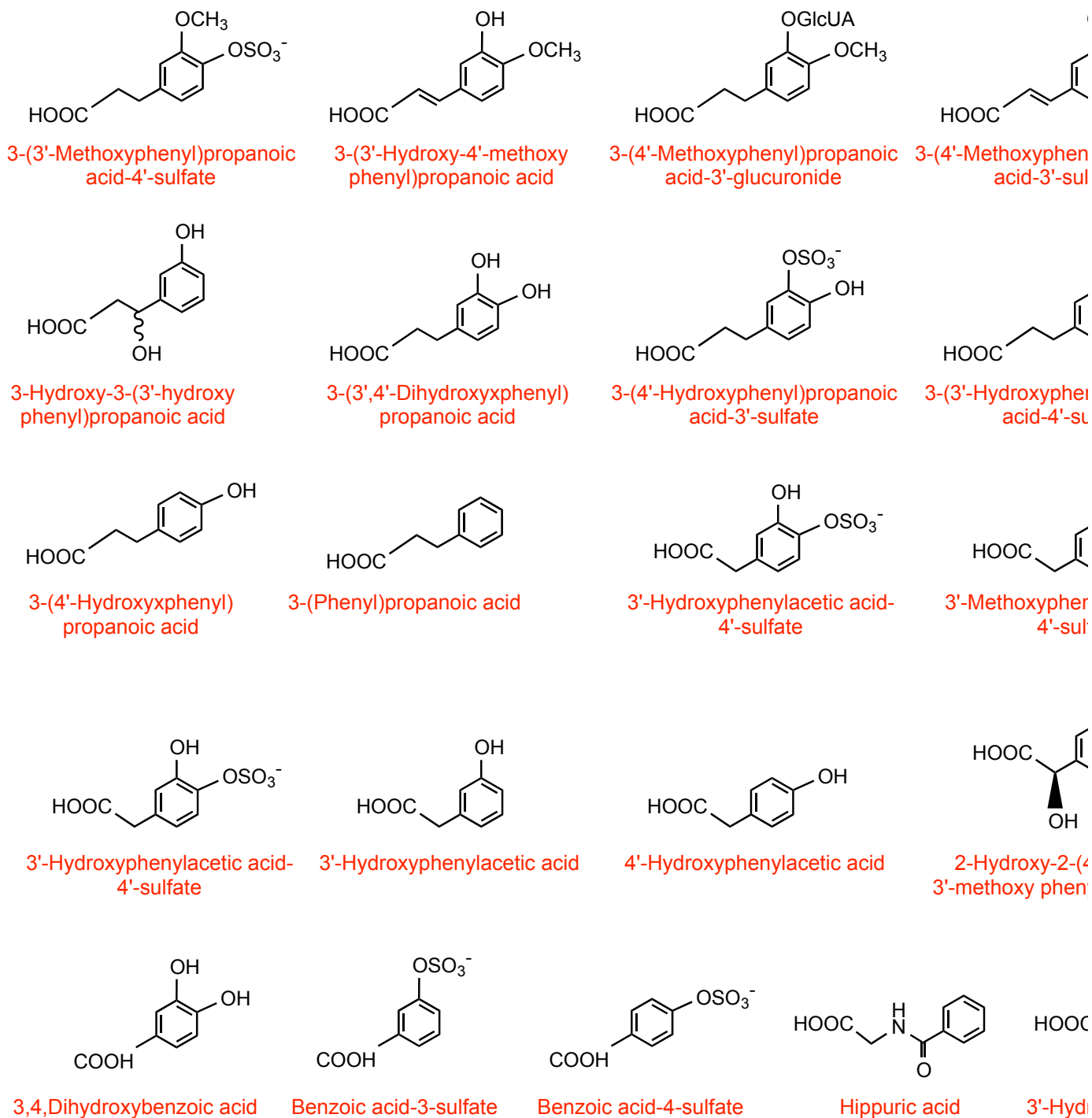Plasma pharmacokinetics of flavanone metabolites after the consumption of 500 mL of orange juice by 10 endurance trained volunteers. The juice contained 398 μmol of (poly)phenols including 330 μmol of flavanones. Data expressed as mean values ± SE (n = 6-10)[a].

Flavanone metabolites	C_{0h} (nmol/L)[a]	C_{max} (nmol/L)	T_{max} (h)	AUC (nmol/h/L)	N
Hesperetin-3´-sulfate	nd	80 ± 24	5.9 ± 0.4	280 ± 62	10
Hesperetin-sulfate	nd	3.3 ± 0.7	6.5 ± 0.3	10 ± 2	6
Hesperetin-sulfoglucuronide	nd	11 ± 2	6.3 ± 0.3	35 ± 8	9
Hesperetin-3´-glucuronide	nd	18 ± 4	6.1 ± 0.3	62 ± 13	10
Hesperetin-7-glucuronide	nd	24 ± 9	5.9 ± 0.4	72 ± 17	10
Hesperetin-3´,5-diglucuronide	nd	2.0 ± 0.6	6.8 ± 0.3	7.2 ± 1.8	9
Hesperetin-3,7´-diglucuronide	nd	1.3 ± 0.4	6.6 ± 0.4	6.4 ± 1.0	7
Hesperetin-5,7-diglucuronide	nd	3.2 ± 1.1	6.8 ± 0.4	15 ± 4	8
Total hesperetin metabolites	**nd**	**143 ± 42[b]**	**6.4 ± 0.4[b]**	**487 ± 109**	–
Naringenin-4´-sulfate	nd	11 ± 7	5.4 ± 0.4	39 ± 16	10
Naringenin-4´-glucuronide	nd	14 ± 2	5.2 ± 0.6	39 ± 7	10
Naringenin-7-glucuronide	nd	21 ± 5	4.2 ± 0.6	93 ± 21	10
Total naringenin metabolites	**nd**	**42 ± 9[b]**	**4.9 ± 0.6[b]**	**171 ± 44**	–

[a] C_{0h} – concentration prior to ingestion of orange juice; C_{max} – peak plasma concentration; T_{max} - time to reach C_{max}; AUC - area under the curve (0-8 h); N – number of volunteers in whose plasma the metabolite was detected; nd – not detected.

[b] The C_{max} and T_{max} values for total hesperetin and naringenin metabolites are significantly different (p<0.001, One-way ANOVA and LSD test).

Table 2
Plasma pharmacokinetics of phenolic acid catabolites after the consumption of 500 mL of orange juice by 10 endurance trained volunteers. The juice contained 398 µmol of (poly)phenols including 330 µmol of flavanones. Data expressed as mean value ± SE (n = 3-10)[a]

Phenolic acid catabolites	C_{0h} (nmol/L)	C_{max} (nmol/L)	T_{max} (h)	AUC (nmol/h/L)	N
Cinnamic acid derivatives					
4'-Hydroxy-3'-methoxycinnamic acid	0.06 ± 0.03	1.5 ± 0.1	1.0 ± 0.2	5.3 ± 0.4	10
3'-Methoxycinnamic acid-4´-glucuronide	0.05 ± 0.02	3.5 ± 0.2	1.7 ± 0.1	13.4 ± 0.7	10
3'-Methoxycinnamic acid-4´-sulfate	0.2 ± 0.1	19 ± 2	1.2 ± 0.2	61 ± 3	10
4'-Methoxycinnamic acid-3´-glucuronide	0.01 ± 0.01	1.3 ± 0.2	6.2 ± 0.2	3.9 ± 0.5	9
3'-Hydroxy-4'-methoxycinnamic acid	nd	tr	–	–	9
Cinnamic acid-4´-glucuronide	nd	tr	–	–	3
3'-Hydroxycinnamic acid-4´-sulfate	nd	tr	–	–	10
4'-Hydroxycinnamic acid-3´-sulfate	nd	tr	–	–	10
Phenylpropanoic acid derivatives					
3-Hydroxy-3-(3'-hydroxy-4'-methoxyphenyl)propanoic acid	0.3 ± 0.1	20 ± 2	7.5 ± 0.1	57 ± 7	10
3-Hydroxy-3-(3'-hydroxyphenyl)propanoic acid	270 ± 44	628 ± 18	3.5 ± 0.2	3202 ± 93	10
3-(3´-Methoxyphenyl)propanoic acid-4´-glucuronide	0.3 ± 0.1	4.7 ± 0.3	6.2 ± 0.2	14 ± 1	10
3-(3´-Methoxyphenyl)propanoic acid-4´-sulfate	0.2 ± 0.1	4.0 ± 0.2	7.2 ± 0.2	11 ± 1	10
3-(3´-Hydroxy-4´-methoxyphenyl)propanoic acid	0.2 ± 0.1	19 ± 1	7.3 ± 0.1	59 ± 5	10
3-(4´-Methoxyphenyl)propanoic acid-3´-glucuronide	nd	6.7 ± 0.6	7.4 ± 0.1	17 ± 2	10
3-(4´-Methoxyphenyl)propanoic acid-3´-sulfate	nd	9.2 ± 0.8	7.4 ± 0.1	25 ± 2	10
3-(3´-Hydroxyphenyl)propanoic acid	0.6 ± 0.2	5.9 ± 0.4	6.0 ± 0.2	22 ± 2	10
3-(4´-Hydroxyphenyl)propanoic acid	8.0 ± 2.0	25 ± 1	5.0 ± 0.2	111 ± 3	10
3-(Phenyl)propanoic acid	0.6 ± 0.3	19 ± 12	6.8 ± 0.1	160 ± 15	10
3-(3´,4´-Dihydroxyphenyl)propanoic acid	nd	tr	–	–	10
3-(3´-Hydroxyphenyl)propanoic acid-4´-sulfate	nd	tr	–	–	10
3-(4´-Hydroxyphenyl)propanoic acid-3´-sulfate	nd	tr	–	–	10
3-(4´-Hydroxy-3´-methoxyphenyl)propanoic acid	nd	tr	–	–	10
Phenylacetic acid derivatives					

36

2-Hydroxy-2-(4′-hydroxyphenyl)acetic acid	1.2 ± 0.3	11 ± 0.6	1.9 ± 0.1	43 ± 2	10
3′-Hydroxyphenylacetic acid	17 ± 5	34 ± 2	3.1 ± 0.2	175 ± 8	10
4′-Hydroxyphenylacetic acid	2 ± 1	151 ± 12	7.2 ± 0.3	607 ± 50	9
2-Hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)acetic acid	0.4 ± 0.1	tr	–	–	10
3′-Hydroxyphenylacetic acid-4´-sulfate	nd	tr	–	–	10
Methoxyphenylacetic acid-glucuronide	tr	tr	–	–	10
4′-Methoxyphenylacetic acid-3´-sulfate	tr	tr	–	–	10
3′-Methoxyphenylacetic acid-4´-sulfate	tr	tr	–	–	10
Benzoic acid derivatives					
3,4-Dihydroxybenzoic acid	tr	tr	–	–	7
Benzoic acid-4-sulfate	tr	tr	–	–	10
Benzoic acid-3-sulfate	tr	tr	–	–	10
Benzoylglycine derivatives					
Hippuric acid	28 ± 8	85 ± 5	6.8 ± 0.4	360 ± 24	10
3′-Hydroxyhippuric acid	7 ± 4	23 ± 1	5.6 ± 0.9	65 ± 4	10
4´-Hydroxyhippuric acid	8 ± 2	58 ± 2	4.0 ± 0.2	224 ± 7	10

[a]C_{ma} – peak plasma concentration; T_{max} - time to reach C_{max}; AUC - area under the curve (0-8 h); N – number of volunteers in whose plasma the metabolite was detected; tr – trace levels; nd – not detected

Supporting Information

Plasma pharmacokinetics of (poly)phenol metabolites and catabolites after ingestion of orange juice

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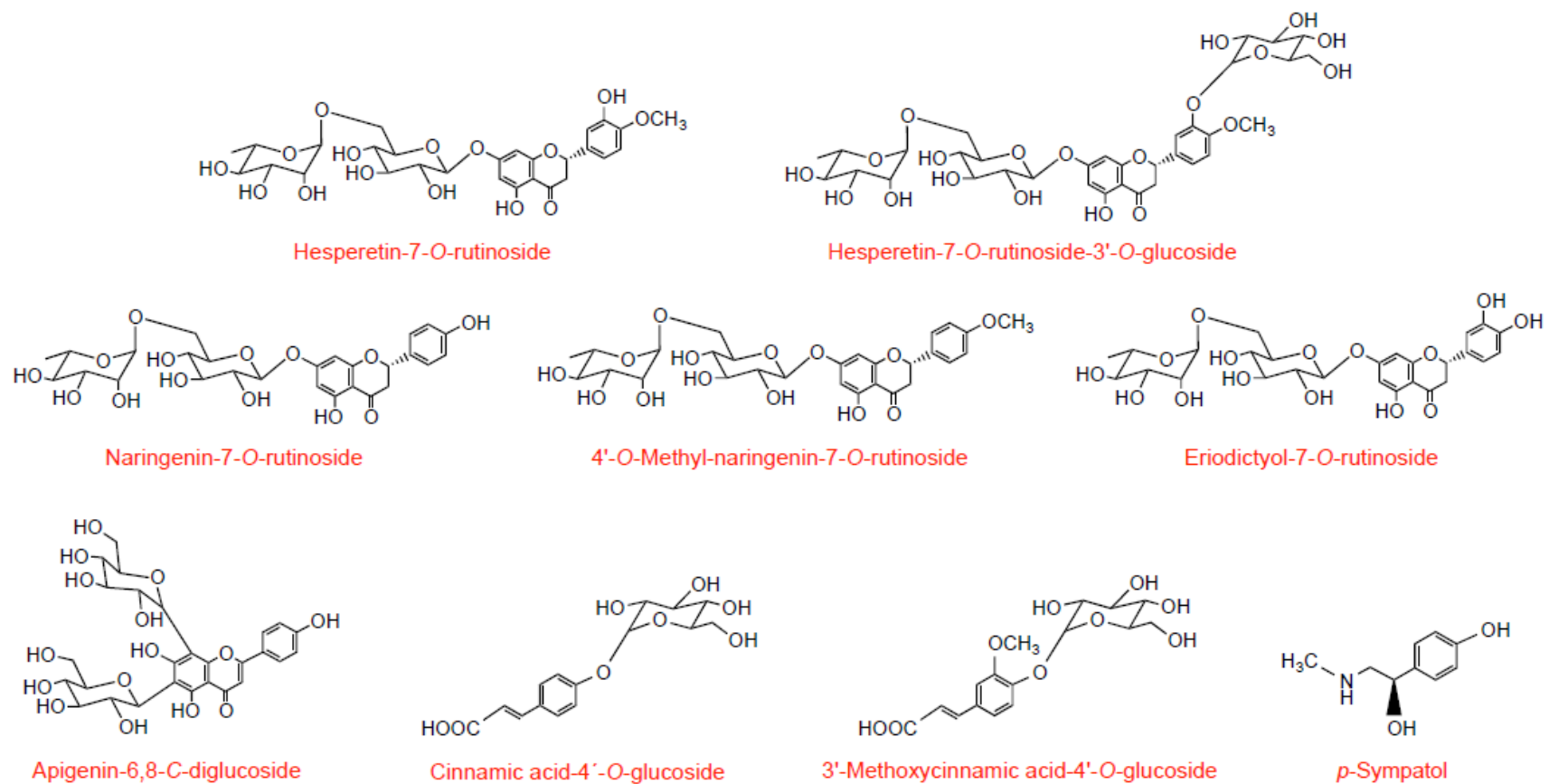*E-mail address:* mariag.pereira@juntadeandalucia.es

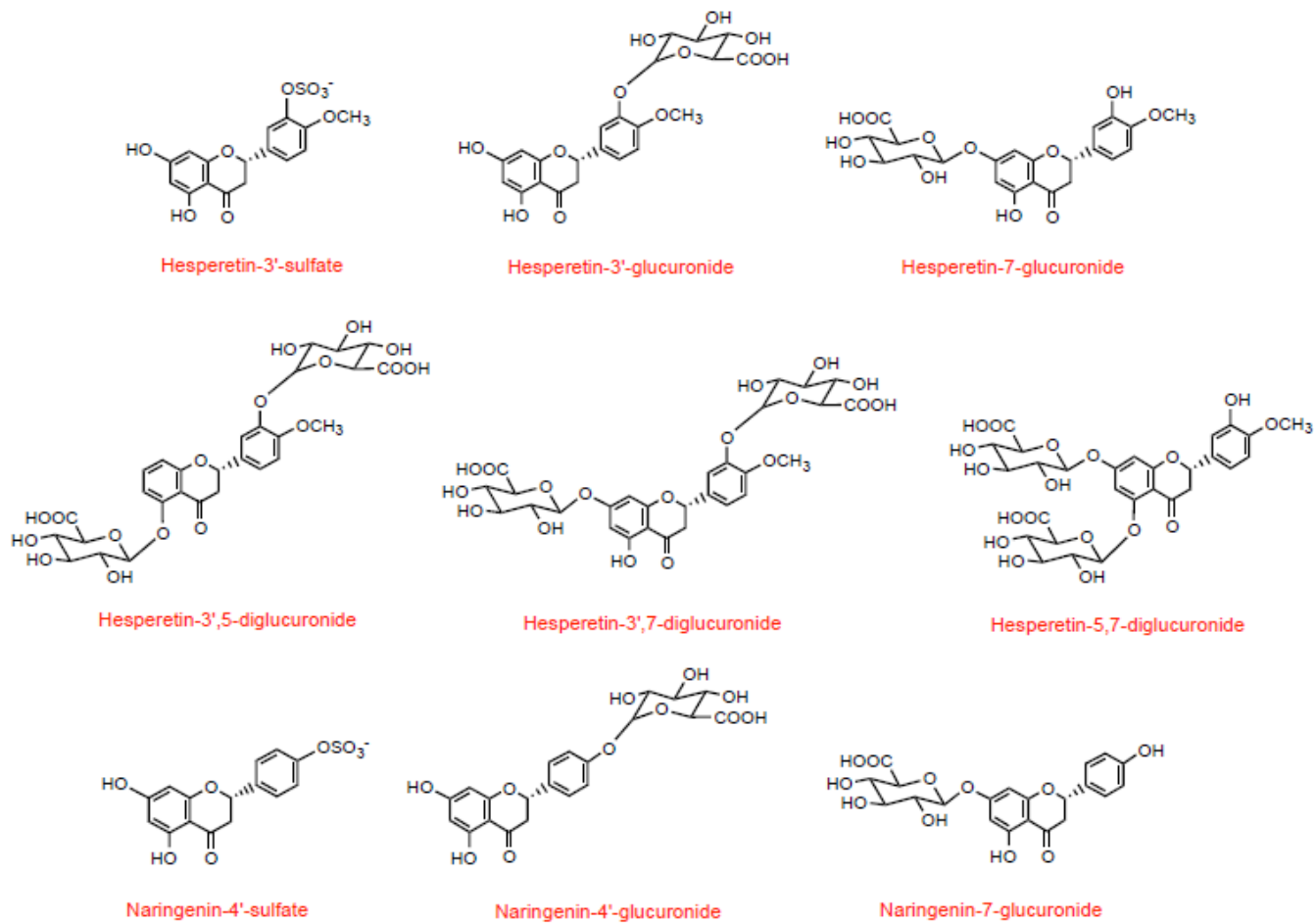**Figure S1.** Structures of orange juice (poly)phenols and *p*-sympatol.

Figure S2. Structures of flavanone phase II metabolites detected in plasma after orange juice intake.

Table S1.

Quantities of flavanone metabolites excreted in urine 0-24 h after the ingestion of 500 mL of orange juice by 10 trained volunteers. The 500 mL of orange juice contained 398 µmol of (poly)phenols.[a]

Flavanone metabolites	(µmol)
Hesperetin-3´-sulfate	1.7 ± 1.0
Hesperetin-sulfate	0.2 ± 0.1
Hesperetin-sulfoglucuronide	0.08 ± 0.04
Hesperetin-3´-glucuronide	6.0 ± 3.6
Hesperetin-5-glucuronide	0.06 ± 0.03
Hesperetin-7-glucuronide	0.8 ± 0.6
Hesperetin-3´,5-diglucuronide	0.3 ± 0.2
Hesperetin-3´,7-diglucuronide	0.02 ± 0.02
Hesperetin-5,7-diglucuronide	0.3 ± 0.3
Total hesperetin metabolites	**9.5 ± 5.8** *(3.8%)*
Naringenin-4´-sulfate	0.1 ± 0.1
Naringenin-sulfoglucuronide	0.02 ± 0.01
Naringenin-4´-glucuronide	1.8 ± 1.0
Naringenin-7-glucuronide	2.0 ± 1.0
Naringenin-4´,5-diglucuronide	0.09 ± 0.06
Naringenin-4´,7-diglucuronide	0.02 ± 0.01
Naringenin-5,7-diglucuronide	0.10 ± 0.09
Total naringenin metabolites	**4.1 ± 2.2** *(5.4%)*
Eriodictyol metabolites	
Eriodictyol-sulfate	0.1 ± 0.1
Eriodictyol-glucuronyl-sulfate	0.1 ± 0.1
Total eriodictyol metabolites	**0.2 ± 0.2** *(5.0%)*
Total flavanone metabolites	**13.8 ± 4.2** *(4.2%)*

[a] The orange juice contained 330 µmol of flavanones (76 µmol naringenin-*O*-glycosides, 250 µmol hesperetin-*O*-glycosides, 4 µmol eriodictyol-7-*O*-rutinoside).

Data are presented in µmol as mean values ± SE (n=10) and in bold italics as a percentage of intake.

Based on data of Pereira-Caro et al. Am. J. Clin. Nutr. 106 (2017) 791–800.

Table S2.

Total quantities of the main phenolic and aromatic compounds excreted in urine 0-24 h after the ingestion of 500 mL of orange juice by 10 trained volunteers. The 500 mL of orange juice contained 398 µmol of (poly)phenols.[a]

Phenolic catabolites	(µmol)
Cinnamic acid derivatives	
3'-Methoxycinnamic acid-4´-glucuronide	0.7 ± 0.2
3'-Methoxycinnamic acid-4´-sulfate	7.8 ± 2.5
4'-Methoxycinnamic acid 3´-glucuronide	1.6 ± 0.5
Minor cinnamic acids	0.16 ± 0.2
Total cinnamic acid derivatives	**10.3 ± 3.3**
Phenylpropanoic acid derivatives	
3-Hydroxy-3-(3´-hydroxyphenyl)propanoic acid	0.2 ± 0.3
3-Hydroxy-3-(3'-hydroxy-4'-methoxyphenyl)propanoic acid	18 ± 5.8
3-(3´-Methoxyphenyl)propanoic acid-4´-glucuronide	1.3 ± 0.4
3-(3´-Methoxyphenyl)propanoic acid-4´-sulfate	2.6 ± 0.8
3-(4´-Methoxyphenyl)propanoic acid-3´-glucuronide	6.3 ± 2.0
3-(4´-Methoxyphenyl)propanoic acid-3´-sulfate	3.2 ± 1.0
Minor phenylpropanoic acids	2.0 ± 0.7
Total phenylpropanoic acid derivatives	**34 ± 11**
Phenylacetic acid derivatives	
2-Hydroxy-2-(4'-hydroxyphenyl)acetic acid	1.8 ± 0.6
2-Hydroxy-2-(3'-methoxy-4'-hydroxyphenyl)acetic acid	0.3 ± 0.1
4´-Hydroxyphenylacetic acid	104 ± 24
Minor phenylacetic acids	3.2 ± 1.0
Total phenylacetic acid derivatives	**109 ± 25**
Benzoic acid derivatives	

Phenolic catabolites		(µmol)
4-Hydroxybenzoic acid		0.4 ± 0.1
Benzoic acid-4-sulfate		1.3 ± 0.3
	Total benzoic acid derivatives	**1.7 ± 0.4**
Benzenetriols		
Benzene-1,3,5-triol		14 ± 6
	Total benzenetriols	**14 ± 6**
Benzoylglycine derivatives		
Hippuric acid		27 ± 8.6
3´-Hydroxyhippuric acid		0.9 ± 0.5
4´-Hydroxyhippuric acid		4.5 ± 1.3
	Total benzoylglycine derivatives	**32 ± 11**
	Total phenolic acid catabolites	**202 ± 54**
	Total excretion as a % of (poly)phenol intake	***51 ± 14***

[a]Phenolic content of baseline urine collected for 12 h prior to orange juice intake used, on an excretion per hour basis, to subtract from excretion values obtained 0-24 h after supplementation to estimate increases in the phenolic content.

Data are presented in µmol as mean values ± SE (n=10) and in bold italics as a percentage of intake.

Based on data of Pereira-Caro et al. Am. J. Clin. Nutr. 106 (2017) 791–800.