Acute effect of oat β-glucan on the bioavailability of orange juice flavanones

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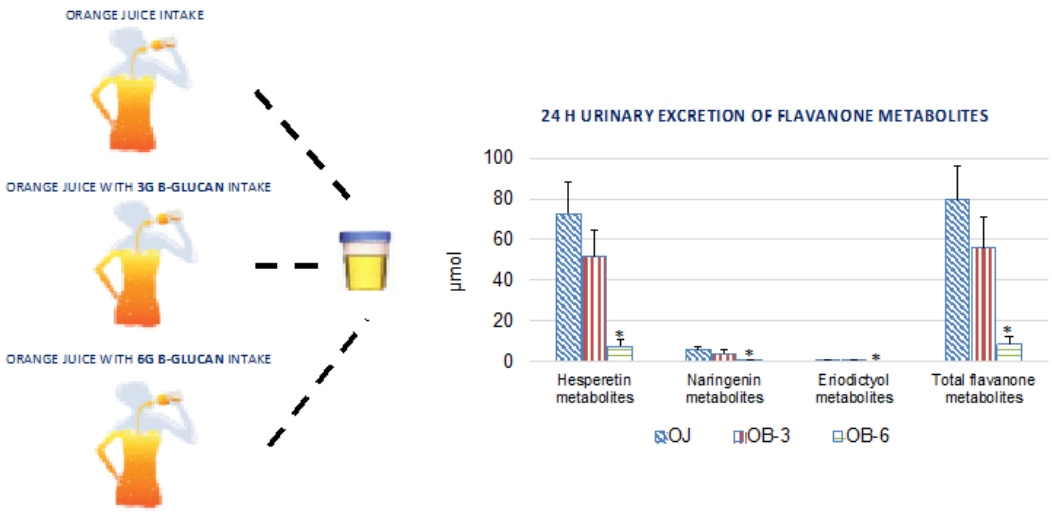
ABSTRACT

The impact of β-glucan on the bioavailability of orange juice (OJ) flavanones was investigated in a randomised controlled trial. Volunteers consumed 500 mL of OJ without or with either 3 g (OB-3) or 6 g (OB-6) of β-glucan. Urine samples, collected 12 h prior to and over a 0-24 h period post-supplementation, were analysed by ultra high-performance liquid chromatography-high resolution mass spectrometry. The overall 0-24 h urinary excretion of the 17 flavanone metabolites identified and quantified in urine after OJ ingestion corresponded to 29.7 µmol, and to 25.0 µmol and 9.3 µmol, respectively, after OB-3 and OB-6 intake. This corresponds to 9.3, 7.9 and 2.9 % recoveries of the 318 µmol of the ingested flavanones. The acute ingestion of OJ with 6 g, but not 3 g of β-glucan lead to a significant reduction ($P < 0.05$) in the excretion of flavanone metabolites compared with consumption of OJ alone.

Keywords

OJ flavanones, bioavailability, UHPLC-HR-MS analysis, oat β-glucan, flavanone metabolites

Graphical Abstract



Introduction

Increasing fruit and vegetable intake is recommended for weight management and prevention of metabolic diseases (Ledoux et al. 2011). Consuming fruit juices is an ready alternative to boost fruit intake (Drewnowski and Rehm 2015). Citrus juices, in particular orange juice (OJ), have been associated with beneficial effects including decreased inflammation and improved lipid profiles (Ledoux et al., 2011). OJ is a rich source of vitamin C and other bioactive components including flavanones (Crozier et al. 2006) which could mediate these effects (Coelho et al. 2013).

However, current research into the health effects of fruit juice consumption has presented some conflicting conclusions. Drinking OJ on a daily basis for 4 weeks has shown to produce increases in postprandial glucose and insulin (Azzini et al. 2017) which may promote insulin resistance. In contrast, daily intake of OJ has been reported to improve glucose and insulin sensitivity in healthy women (Lima et al., 2019). None-the-less, consideration of nutritional strategies to attenuate glucose and insulin responses following OJ consumption remains of importance. One strategy to counteract the glucose spike following fruit juice consumption is to combine intake with dietary fibre. In a controlled double-blind cross-over trial, Dong et al. (2016) evaluated the effects of OJ intake with different fibre concentrations on post-prandial glycemia, with overweight men consuming either OJ with 5.5 g of added orange pomace fibre, freshly squeezed OJ, or an isocaloric sugar matched control alongside a high fat, high carbohydrate breakfast. They found that the added orange pomace fibre significantly reduced the maximum change in glucose concentration (1.9 ± 0.21 mmol/L) compared to the other groups (2.3–2.4 mmol/L), and delayed the time taken to reach maximum glucose concentration, after both breakfast and lunch.

In this context, the bioavailability of flavanones present in OJ is an important factor that needs to be considered when studying effects of β-glucan supplementation. In the

current study the 24 h-urinary excretion of OJ flavanone metabolites by healthy male and female volunteers was determined following the consumption of OJ alone, and OJ with 3 g and 6 g oat β-glucan.

Materials and methods

Chemicals

The flavanone metabolites hesperetin-7-glucuronide, hesperetin-3′-sulfate, naringenin-4′-glucuronide, and naringenin-7-glucuronide were obtained from Toronto Research Chemicals (Toronto, Canada). Hesperetin-7-*O*-rutinoside (hesperidin), 4′-methoxy-naringenin-7-*O*-rutinoside (didymin), and naringenin-7-*O*-rutinoside (narirutin) were obtained from Extrasyntheses (Genay, France). 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).

Study Design

Participants aged between 20 and 38 years with a BMI of 26.4 ± 1.1 kg/m^2 (range 22.2-33.2 kg/m^2) volunteered to participate in the study. All were non-smokers, with a stable weight for the previous 3 months, and not engaged in regular strenuous physical activity. Other exclusion criteria included suffering from chronic disease, taking any medication, or following a special diet, including being vegetarian and vegan. 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 (NCT04867655).

Ten volunteers participated in each of the three 24-h feeding trials. On the morning of the trial participants consumed 500 mL of OJ (Tropicana 'with bits', purchased from a local supermarket) without or with OatWell fibre supplement (CreaNutrition) purchased from Holland and Barrett (Byers Road, Glasgow, UK) providing either 3 g or 6 g of β-glucan.

Feeding those subjects who participated in more than one arm of the trial was separated by a wash out period of 7 days. Urine was collected 12 h prior to (baseline) and over a 24 h period after consumption of the OJ alone and OJ with a β-glucan supplement. Urine was collected in sealable flasks kept on ice. The total volume of each urine fraction was recorded, and 2 mL aliquots were stored at –80 °C prior to analysis. Participants were asked to follow a special low (poly)phenol diet and record weighed dietary intake for 2 days preceding each trial and during the day of the experimental trial.

Extraction and analysis of orange juice and urine samples

The pulp-enriched OJ used in the feeding study was extracted using a previously published protocol (Pereira-Caro et al. 2020a). Briefly, 5 mL aliquots of juice, 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, dissolved in 6 mL of 50% aqueous methanol and stored at −80 °C before being analysed. Urine samples were defrosted, vortexed and centrifuged at 16000*g* for 15 min at 4 °C prior to the analysis of 5 μL aliquots by HPLC-HR-MS.

Urine, OJ and reference compounds were analysed in triplicate with the procedures described by Pereira-Caro et al. (2017) with a Dionex Ultimate 3000 Rapid Separation ultra-HPLC system comprising an ultra-HPLC pump, a photodiode array detector scanning from 200 to 600 nm, and an autosampler operating at 4 °C (Thermo Fisher Scientific, San José, CA, USA). Reverse-phase separations were carried out using a 150 x 4.6-mm, 5 μm, 100 Å C18 Kinetex column (Phenomenex, Torrance, CA, USA) maintained at 40°C and eluted at a flow rate of 1.0 mL/min with a 45-min gradient of 3-50% of methanol in 0.1% aqueous formic acid. After passing through the flow cell of the photodiode array detector, the column eluate was split and 0.2 mL/min was directed to an Exactive Orbitrap mass spectrometer fitted with a heated electrospray ionization probe (Thermo Fisher Scientific)

operating in negative ionization mode. Analyses were based on scanning from 100 to 1000 *m/z*, with in-source collision-induced dissociation at 25.0 eV. The capillary temperature was 300 °C, the heater temperature was 150 °C, the sheath gas and the auxiliary gas flow rate were both 20 U, the sweep gas was 3 U, and the spray voltage was 3.00 kV. Data acquisition and processing were carried out with the use of Xcalibur 3.0 software.

Identification and quantification of OJ (poly)phenols and their flavanone metabolites was achieved as described previously (Pereira-Caro et al. 2017).

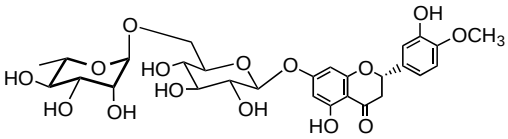
Statistical analysis

Data were assessed for normality of distribution with the use of the Shapiro-Wilk test and revealed that data were not normally distributed. Kruskal-Wallis One-Way ANOVA test was used to determine whether differences in total and relative excretion of flavanone metabolites were significantly different bewtwwen OJ and the OJ plus β-glucan trials. $P < 0.05$ was considered significant, and data are presented as means ± SEs. Statistical analyses were performed with the use of Statistica (version 10.0; StatSoft Inc.).

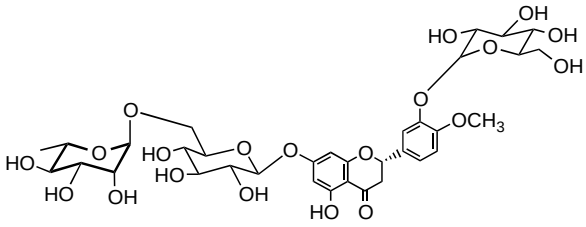
Results

Identification and quantification of OJ polyphenols

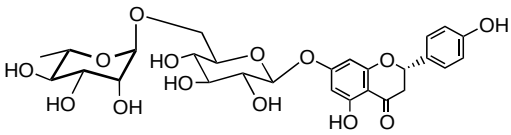
The 500 mL OJ consumed by the volunteers contained hesperetin-7-*O*-rutinoside (**1**) (204 μmol), hesperetin-7-*O*-rutinoside-3′-*O*-glucoside (**2**) (1 μmol), naringenin-7-*O*-rutinoside (**3**) (67 μmol), 4′-methoxy-naringenin-7-*O*-rutinoside (**4**) (41 μmol), eriodictyol-7-*O*-rutinoside (**5**) (4.8 μmol), apigenin-6,8-*C*-diglucoside (**6**) (16 μmol) and 3′-methoxycinnamic acid-4′-*O*-glucoside (aka ferulic acid-4′-*O*-glucoside) (**7**) (34 μmol). In total, the ingested juice contained 368 μmol (poly)phenols, of which 318 μmol were flavanones.



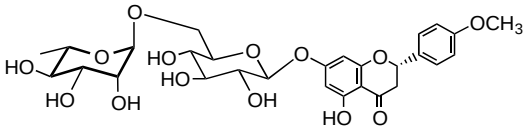
Hesperetin-7-*O*-rutinoside (**1**)



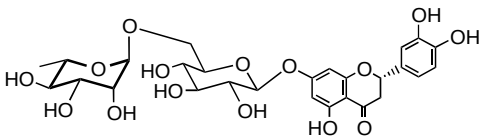
Hesperetin-7-*O*-rutinoside-3'-*O*-glucoside (**2**)



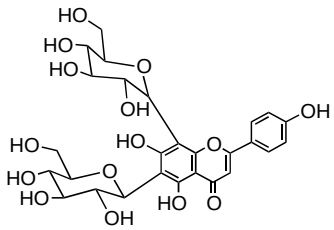
Naringenin-7-*O*-rutinoside (**3**)



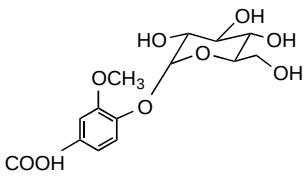
4'-Methoxy-naringenin-7-*O*-rutinoside (**4**)



Eriodictyol-7-*O*-rutinoside (**5**)



Apigenin-6,8-*C*-diglucoside (**6**)

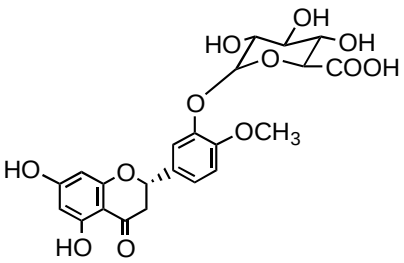


3'-Methoxycoumaric acid-4´-*O*-glucoside (**7**)

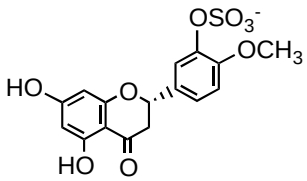
Urinary excretion of flavanone metabolites

A total of 17 flavanone metabolites were identified and quantified in urine collected 0-24 h after OJ consumption as outlined in previous publications (Pereira-Caro et al., 2016; 2017). Quantitative data on the urinary excretion of flavanone metabolites are summarized in Figure 1. In general terms, there was no statistically significant difference in the excretion of hesperetin, naringenin or eriodictyol metabolites when volunteers consumed OJ or OJ with 3 g. However, co-ingestion of OJ with 6 g of β-glucan significantly reduced flavanone excretion.

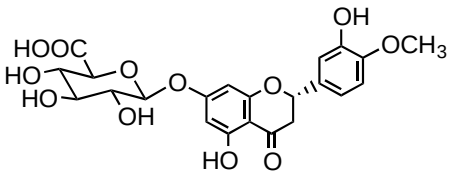
The levels of individual flavanone metabolites in urine 0-24 h after OJ or OJ with 3 g of β-glucan (OB-3) or OJ with 6 g of β-glucan (OB-6) are summarized in (Table 1). Hesperetin metabolites were excreted in highest quantities (Figure 1). Eight hesperetin metabolites were detected with the main component being hesperetin-3´-glucuronide (**8**), a reported urinary biomarker of OJ consumption (Pereira-Caro et al. 2017), followed by hesperetin-3´-sulfate (**9**), hesperetin-7´-glucuronide (**10**) and a hesperetin-glucuronyl-sulfate (Table 1). Among the six naringenin metabolites, naringenin-4´-glucuronide (**11**) was present in highest amounts followed by its isomer naringenin-7-glucuronide (**12**). Lower quantities of an eriodictyol-glucuronide and an eriodictyol-sulfate were also detected. The overall 0-24 h excretion of flavanone metabolites corresponded to 29.7 ± 4.3 µmol, 25.0 ± 6.7 µmol and 9.3 ± 2.3 µmol after OJ, OB-3 and OB-6 intake, respectively, which represent a recovery of 9.3%, 7.9 % and 2.9 % of the 318 µmol of the flavanones ingested.



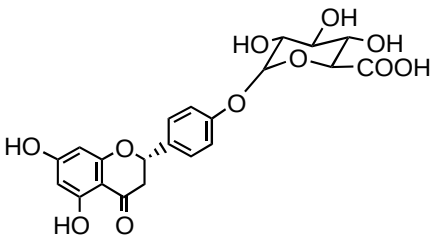
Hesperetin-3'-glucuronide (**8**)



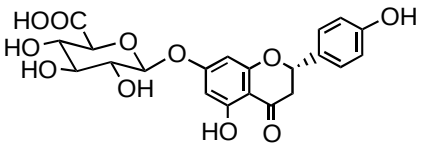
Hesperetin-3'-sulfate (**9**)



Hesperetin-7-glucuronide (**10**)



Naringenin-4'-glucuronide (**11**)



Naringenin-7-glucuronide (**12**)

It is of note that there a clear statistically significant decrease in the total excretion of flavanone metabolites in urine after OJ consumption with 6 g, but not 3 g, of β-glucan intake (Table 1). Indeed, the co-ingestion of 6 g of β-glucan with OJ resulted in significant 79%, 86% and 85% decreases, respectively, in the excretion of naringenin, hesperetin and eriodictyol metabolites compared with the ingestion of OJ on its own.

Discussion

This study evaluated the effect of a supplementation with 3 g or 6 g of soluble fibre on the bioavailability of OJ flavanones in healthy adults. The 0-24 h urinary excretion of flavanone metabolites was in line with previously published studies (Borges et al. 2013; Pereira-Caro et al. 2014; 2015; 2017). In the current investigation, the ingestion of OJ resulted in a 0-24 h urinary excretion of flavanone metabolites corresponding to 12.1% of flavanones intake. Co-ingestion of OJ with 3 g of β-glucan did not have a significant impact on excretion of the flavanones metabolites (Table 1). However ingestion of the juice with 6 g of β-glucan resulted in a significant 3.1-fold reduction (9.3 μmol *versus* 29.7 μmol) in the excretion of the metabolites (Table 1). This marked effect could be because, as a soluble fibre, β-glucan increases viscosity, which reduces the rate of absorption of flavanones from the gastrointestinal (GI) tract by delaying gastric emptying, (Malkki et al. 2001).

While β-glucan-induced delayed of the absorption of glucose brings about favourable effects on post-prandial glycemia (Dong et al. 2016), it has been suggested that dietary fibre may also reduce the bioavailability of some beneficial compounds, including polyphenols, via the formation of an unstirred layer adjacent to the mucosa of the intestinal wall, and this layer would act as a physical barrier to the absorption of nutrients (Bohn 2014). In an earlier study, Tew et al. (1996) demonstrated that addition of 40 g of

wheat bran fibre to a control meal containing 15 g of dietary fibre resulted a 55% reduction in plasma genistein ($P < 0.05$) and a reduction of 20% in total urinary genistein ($P < 0.03$) in seven healthy women. This is a smaller effect on urinary excretion than observed in the current study with OJ flavanones and 6 g of β-glucan.

While some of the OJ flavanones are absorbed in the upper GI tract, ~70 % of absorption occurs in the lower bowel (Borges et al. 2013; Pereira-Caro et al. 2020b). OJ flavanones coming into contact with β-glucan reduced the amount available for absorption in the GI tract and in the process might have increased the level of flavanones available for breakdown to phenolic catabolites by the colonic microflora. This could impact on bioactivity. It has been hypothesized that carrying dietary polyphenols to the lower parts of the GI tract could be one of the essential functions of dietary fibres (Saura-Calixto 2011). The effect of β-glucan addition to OJ on the subsequent excretion of phenolic catabolites remains to be investigated.

Conclusions

This work represents a preliminary study evaluating the effect of β-glucan consumption on the bioavailability of OJ polyphenols in healthy adults, with special emphasis on the urinary excretion of the phase II flavanone metabolites, notably glucuronide and sulfate derivatives. The acute co-ingestion of 6 g of β-glucan and OJ may affect the bioavailability of OJ polyphenols by decreasing their absorption in GI tract. However, further investigations are needed to determine the impact of β-glucan intake on plasma levels and urinary excretion of OJ metabolites and gut-derived phenolic acid catabolites.

Acknowledgements

Our thanks to the volunteers who participated in the study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

The data that support the findings of this study are available from the corresponding author, [A.L.G], upon reasonable request.

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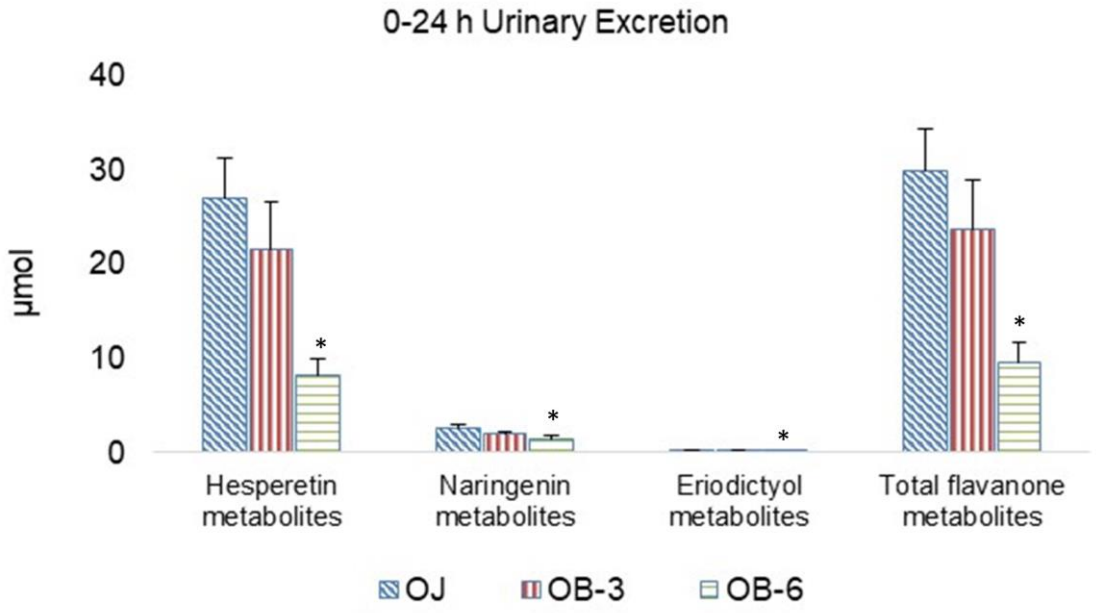

Figure 1. Excretion of flavanone metabolites 0–24 h after the ingestion of orange juice (OJ), and OJ with 3 g (OB-3) and 6 g (OB-6) of soluble fibre by ten volunteers. Values are expressed as means ± SEs. The y-axis is the urinary excretion expressed in µmol. * Values that decreased significantly after OJ supplementation with β-glucan ($P < 0.05$) were obtained by using Kruskal-Wallis One-Way ANOVA test.

Table 1. Urinary excretion of flavanone metabolites 0-24h after ingestion of 500 mL of orange juice (OJ), and 500 mL of OJ with 3 g of β-glucan (OB-3) and 6 g of β-glucan (OB-6). Data expressed as mean values in μmol ± SE (n=10) and in parentheses as a percentage of intake. In total the ingested juice contained 368 μmol of (poly)phenols of which 318 μmol were flavanones.

Flavanone metabolites	OJ	OB-3	OB-6
Naringenin metabolites			
Naringenin-4′,7-diglucuronide	0.01 ± 0.01	0.01 ± 0.01	0.001 ± 0.00*
Naringenin-5,7-diglucuronide	0.02 ± 0.01	0.03 ± 0.01	0.002 ± 0.001*
Naringenin-4′,5-diglucuronide	0.01 ± 0.01	0.02 ± 0.01	0.003 ± 0.001*
Naringenin-glucuronyl-sulfate	0.01 ± 0.01	0.02 ± 0.01	0.001 ± 0.001*
Naringenin-4′-glucuronide	1.4 ± 0.2	1.6 ± 0.4	0.6 ± 0.1*
Naringenin-7-glucuronide	1.2 ± 0.1	1.1 ± 0.2	0.7 ± 0.2*
Total naringenin metabolites	2.6 ± 0.3 (2.4%)	2.8 ± 0.6 (2.6%)	1.3 ± 0.3 (1.2%)*
Hesperetin metabolites			
Hesperetin-3′,7-diglucuronide	0.17 ± 0.06	0.09 ± 0.02	0.06 ± 0.02*
Hesperetin-5,7- diglucuronide	0.9 ± 0.3	1.0 ± 0.3	0.12 ± 0.03*
Hesperetin-3′,5- diglucuronide	0.3 ± 0.1	0.6 ± 0.2	0.05 ± 0.02*
Hesperetin-*O*-glucosyl-sulfate	0.3 ± 0.1	0.4 ± 0.1	0.07 ± 0.02*
Hesperetin-glucuronyl-sulfate	1.8 ± 0.8	1.8 ± 0.7	0.2 ± 0.1*
Hesperetin-7-glucuronide	3.0 ± 0.5	3 ± 1	0.8 ± 0.1*
Hesperetin-3′-glucuronide	16 ± 2	10 ± 3	5 ± 1*
Hesperetin-3′-sulfate	4.6 ± 0.7	5 ± 1	1.8 ± 0.6*
Total hesperetin metabolites	27 ± 4 (13.2%)	22 ± 6 (10.7%)	8 ± 2 (3.9%)*
Eriodictyol metabolites			
Eriodictyol-glucuronide	0.01 ± 0.01	0.01 ± 0.01	0.001 ± 0.000*
Eriodictyol-sulfate	0.12 ± 0.03	0.12 ± 0.08	0.02 ± 0.01*
Total eriodictyol metabolites	0.13 ± 0.04 (2.7%)	0.13 ± 0.09 (2.7%)	0.02 ± 0.01 (0.4%)*
Total flavanone metabolites	29.7 ± 4.3 (9.3%)	25.0 ± 6.7 (7.9%)	9.3 ± 2.3 (2.9%)*

*Values that are significantly lower ($P < 0.05$) after OJ supplementation with β-glucan compared with OJ alone (Kruskal-Wallis One-Way ANOVA test).