White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/160668/

Version: Accepted Version

Article:
Li, L, Lyall, GK, Martinez-Blazquez, JA et al. (2020) Blood orange juice consumption increases flow-mediated dilation in adults with overweight and obesity: A randomized controlled trial. The Journal of Nutrition, 150 (9). pp. 2287-2294. ISSN: 0022-3166

https://doi.org/10.1093/jn/nxaa158

Copyright © The Author(s) on behalf of the American Society for Nutrition 2020. This is an author produced version of a paper published in The Journal of Nutrition. Uploaded in accordance with the publisher's self-archiving policy.

Reuse
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Blood orange juice consumption increases flow-mediated dilation in adults with overweight and obesity: A randomized controlled trial

Lu Li[1,2], Gemma K. Lyall[3], J. Alberto Martinez-Blazquez[4], Fernando Vallejo[4], Francisco Tomas-Barberan[4], Karen M. Birch[3], and Christine Boesch[2]

[1]Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing 100048, China; [2]School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom; [3]School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom; and [4]CEBAS-CSIC, Quality, Safety and Bioactivity of Plant Foods, Murcia, Spain.

Corresponding authors: C Boesch, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, United Kingdom. Tel +44 113 3430268. Email: c.bosch@leeds.ac.uk and K Birch, School of Biomedical Sciences, University of Leeds, +44 113 3436713, k.m.birch@leeds.ac.uk

A list of all authors' last names: Li, Lyall, Martinez-Blazquez, Vallejo, Tomas-Barberan, Birch, and Boesch.

Word count: 3972

Number of figures: 3

Number of tables: 2

Supplementary data: none

Running title: Blood orange juice and endothelial function

Abbreviations: ALDH2, aldehyde dehydrogenase; AUCpeak, area under the shear rate curve to peak dilation; BOJ, blood orange juice; CD, control drink; cGMP, cyclic guanosine monophosphate; CHD, coronary heart disease; CVD, cardiovascular disease; DBP, diastolic

blood pressure; ET-1, endothelin 1; FMD, flow-mediated dilation; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LDL, low-density lipoprotein; LPH, lactase phlorizin hydrolase; NO, nitric oxide; RCT, randomized controlled trial; RSD, relative standard deviation; SBP, systolic blood pressure; TNF-α, tumour necrosis factor-α; UHPLC-QqQ-MS, ultra-high performance liquid chromatography coupled with triple-quadrupole mass spectrometry; VTI, velocity time integral.

Financial support: This work was supported by China Scholarship Council-University of Leeds Scholarship.

Author disclosures: LL, GKL, JAMB, FV, FTB, KMB, and CB, no conflicts of interest.

Clinical Trial Registry number NCT03611114, registered at clinicaltrials.gov (with information on the full protocol); Data described in the manuscript, code book and analytical code will be made available upon request pending (application and approval, payment, other).

Abstract

Background: Epidemiological studies have indicated an inverse association between citrus fruit consumption and cardiovascular disease (CVD) risk. There is, however, a paucity of data concerning effects of blood orange juice (BOJ) intake on endothelial function and cardiovascular risk biomarkers.

Objective: We examined short-term effects of BOJ on endothelial function, blood pressure, lipid profile, and inflammatory markers in healthy participants of European origin who were overweight or obese.

Methods: In a randomized controlled single-blind crossover trial, 15 men and women (age: 28.7 ± 6.5 y; BMI: 28.3 ± 3.1 kg/m^2) consumed BOJ or a sugar-matched control drink (CD) (200 mL twice daily) for 2 weeks with a washout period of 1 week. Endothelial function, measured as flow-mediated dilation (FMD) (primary outcome), and the following secondary outcomes blood pressure, anthropometric measures, lipid profile, inflammatory markers, markers of vasodilation and vasoconstriction, and urinary flavanone metabolites were evaluated prior to and at the end of each treatment period following an overnight fast. Changes between treatments over time were assessed using repeated-measures ANOVA.

Results: The results demonstrate a significant increase in FMD following BOJ consumption (pre: 8.15% ± 2.92%, post: 10.2% ± 3.31%, P=0.002) compared to CD (pre: 8.11%± 2.52%, post: 7.77%± 2.43%) (time by treatment interaction: P=0.001). Concurrent significant increases in urinary hesperetin-3'-glucuronide and hesperetin-7-glucuronide were observed following BOJ supplementation only (time by treatment interaction: $P \leq 0.01$). Baseline blood pressure, lipid profile, hsCRP, and ET-1 were generally within healthy ranges and unaffected by the intervention.

Conclusions: A two-week consumption of BOJ exerts favourable effects on endothelial function in healthy women and men who were overweight or obese, which is likely mediated by the combined actions of anthocyanin and flavanone metabolites on mechanisms that contribute to enhancing NO bioavailability. This trial was registered at clinicaltrials.gov as NCT03611114.

29

30 **Keywords**: flavanones, blood orange juice, overweight/obese participants, endothelial
31 function, flow-mediated dilation, shear rate, urinary flavanone metabolites

32

Introduction

Epidemiological studies have suggested that a higher intake of citrus fruit is associated with reduced risk of ischemic stroke (1), lower levels of inflammation and endothelial dysfunction (2). Bioactives in citrus fruits such as hesperidin and naringin have received considerable attention due to *in vitro* and *in vivo* evidence demonstrating anti-atherogenic effects (3, 4). Given the role of inflammation and endothelial dysfunction in the development of atherosclerosis and that endothelial function is a strong prognostic indicator for cardiovascular events (5, 6), the consumption of citrus fruit may have significant impact.

However, evidence from randomized controlled trials (RCTs) investigating effects of orange juice on endothelial function and inflammatory markers is conflicting (7-9). A number of factors have been highlighted which may contribute to observed heterogeneity including differences in trial design, food composition, flavonoid source as well as volunteer related (such as age, sex, ethnicity) (10, 11). Indeed, the majority of previous RCTs have involved men or postmenopausal women. Issues associated with the inclusion of premenopausal women mainly relate to fluctuations in either exogenous or endogenous reproductive hormones. The increase in estrogen during the late follicular phase of the menstrual cycle has been seen to markedly increase endothelial function, as measured by flow mediated dilation (FMD) (12, 13). Regulated by an upregulation in nitric oxide (NO), FMD is a direct result of blood flow enhanced shear stress along the endothelium. Thus shear stress as the stimulus for FMD is crucial to the interpretation of FMD data (14) but has rarely been calculated and discussed in the context of polyphenol intervention studies.

Genetic variants have been highlighted as important contributors to inter-individual differences (15). Lactase phlorizin hydrolase (LPH), for example, plays a pivotal role in the exclusive hydrolysis of some polyphenol glucosides prior to absorption (16) with low levels occurring in 5% of European and 90% of African and Asian adults. Variations in the enzyme aldehyde dehydrogenase (ALDH2), which contributes to nitrate conversion into NO (17), are highly prevalent among Asian populations (18) but relatively uncommon in Caucasians (19).

However, details on participant ethnicity are frequently not provided in human intervention studies.

In comparison to blond orange juice, blood orange juice (BOJ) has received much less attention yet it is an abundant source for bioactives such as anthocyanins, flavanones, hydroxycinnamic acids (20), and vitamin C (21). With the exception of Buscemi et al. (22) who showed an increase in FMD in men with moderately increased CVD risk after one-week BOJ consumption, none of the other studies were able to demonstrate beneficial changes in CVD biomarkers after chronic exposure of up to 12 weeks (23, 24).

The aim of this study was to investigate the effects of short term consumption of BOJ on endothelial function and other CVD risk factors. We chose participants who were overweight or obese and of Caucasian heritage as higher BMI and chronic low-grade inflammation are associated with a higher risk of coronary heart disease (CHD) and impaired endothelial function (25-27).

Methods

Study population

Sixteen healthy men and premenopausal women, aged 20-45 y, were recruited according to the following eligibility criteria: Caucasians (of European origin), generally healthy with absence of any form of CVD, non-smokers, BMI over 25 kg/m^2, no medications or dietary supplements (vitamins, antioxidants), absence of lactose intolerance. Fifteen participants completed the study as one participant was excluded due to the use of medication during the intervention. The study was approved by Biological Sciences Faculty Research Ethics Committee, University of Leeds (Ethics Reference Number: BIOSCI 15-030), in accordance with ethical principles of the Declaration of Helsinki. Written informed consent from all participants was obtained prior to study commencement.

Study design

The study design was a randomized, controlled, single-blind, crossover trial (**Figure 1**). During two 2-week periods, participants were asked to consume sugar-matched 400 mL of BOJ or control drink (CD) (200 mL with breakfast and 200 mL with dinner) daily, with a 1-week washout period between each intervention. Block randomization was conducted to allocate drink sequences to participant codes. After enrolment onto the study, participants visited the vascular laboratory at the University of Leeds on four separate occasions for measurements prior to and following each 2-week period (January-June 2017). Participants were instructed to stay fasted and refrain from exercise for 12 h before measurements in the morning. After the participant was supine and comfortable for 15 min to reach a cardiovascular steady state, blood pressure was measured in triplicate with 2-minute intervals. Endothelial function was evaluated via brachial artery FMD with each measurement performed at the same time of the day and on the same area of the brachial artery, as explained below. Following FMD measurements, venous blood samples from the antecubital vein were collected and participants were asked to provide a spot urine sample. Anthropometric measures were conducted at baseline and following each intervention. Participants were asked to maintain their lifestyle as usual throughout the study, including dietary routines and physical activity level and they were asked to record the time of individual drink consumption on a separate sheet which was returned to the researcher after the 2-week periods. Questionnaires on the habitual intake of citrus fruit/juice and other flavonoid sources were also collected. Researchers who conducted the measurements were blinded and only unblinded post data analysis.

Female participants started the intervention on specific days of the menstrual cycle (e.g., day 4 of a 28-day menstrual cycle) in order to avoid any measurements during the late follicular phase. For women consuming the oral contraceptive pill, either as combined estrogen and progesterone or progesterone only, all assessments were undertaken during the period of time the pill was being consumed (not in pill breaks).

Intervention products

Commercially available blood orange juice (BOJ) (47 kcal/dL) and a low flavonoid control drink (CD) (41 kcal/dL) were obtained from Waitrose, Leeds, UK. The flavanone concentration of the juices was analyzed by HPLC-MS (28). Hesperidin and narirutin were 80.2 ± 2.7 and 9.5 ± 0.1 mg/dL for BOJ, and 6.3 ± 0.2 and 1.0 ± 0.1 mg/dL for CD, respectively (both $P<0.001$). The total anthocyanin concentration, analyzed using pH differential method (29), was 2.40 ± 0.13 mg/dL for BOJ with no anthocyanins being detectable in CD. Sugar quantification was performed by Dionex ICS-5000 (30) and the total sugar concentration of BOJ and CD calculated at 14.3 ± 0.6 and 14.7 ± 0.7 g/dL, respectively ($P=0.591$). Test drinks for 2 weeks of consumption were provided to each participant at the start of the intervention and stored in their home refrigerators until consumption.

Endothelial function

The local protocol for assessment of brachial artery endothelial function via FMD (31, 32) was in accordance with established guidelines (14, 33) using duplex ultrasonography (Vivid E9 with XDclear, GE Healthcare, US). Following 15 min of rest in a supine position in a quiet and temperature-controlled vascular laboratory, the brachial artery was imaged above the antecubital fossa in the longitudinal plane. Resting brachial artery diameter was recorded for 20 s at 15 images/s using vascular imaging software (Vascular Imager, Medical Imaging Applications, Iowa, USA). Reactive hyperemia was created by inflating a pneumatic cuff on the forearm for 5 min at 220 mmHg. Post cuff deflation brachial artery diameter and blood flow were recorded for 180 s starting 30 s before cuff deflation. Brachial artery diameter and blood flow were assessed off-line using Brachial Analyzer for Research (version 6, Medical Imaging Applications, Iowa, USA). Peak diameter was calculated from the maximum diameter of the moving averages of 3 consecutive diameters. Absolute FMD (mm; Peak diameter – resting diameter) and relative FMD (%; absolute FMD / resting diameter x 100) were determined. Velocity time integral (VTI) and the area under the shear rate curve (AUC) were calculated as previously described (34). AUC to peak diameter was calculated as an indication of the stimulus for FMD (14). Time to peak diameter was calculated as the time period starting from

cuff deflation to peak diameter. Specifically, relative FMD was the primary outcome and the other measures of FMD (resting diameter, AUCpeak, absolute FMD, scaled FMD index, time to peak diameter) were secondary outcomes. To determine reliability of ultrasound measurements, FMD was conducted on 10 healthy participants with each individual examined twice on two consecutive days. Resting brachial artery diameter showed a coefficient of variation (CV) of 0.4% and CV of relative FMD was 6.12%.

Plasma cardiovascular risk biomarkers

Serum and EDTA plasma were generated using standard procedures. Urine samples were centrifuged (2000 x g at 4°C, 15 min) and filtered (0.22 µm CA-CN syringe filter). Aliquots of serum, plasma and urine were stored at -80°C prior to analysis. Lipid profile, hsCRP and estradiol were analysed in serum using standardized assays by the Pathology Services (Leeds General Infirmary). Plasma ET-1 and cGMP were measured using commercially available immunoassays (R&D Systems, Abingdon, UK).

Urinary metabolite analysis

Major urinary metabolites were analyzed by UHPLC-QqQ-MS as previously described (35). The limit of detection (LOD) was determined as the concentration of analytes with a signal to-noise ratio of at least 3, and the limit of quantification (LOQ) was the lowest standard with a signal-to-noise ratio of at least 10. LOQs were 80 nM for hesperetin, 50 and 80 nM for hesperetin-7-and 3'-glucuronides, respectively, and 40 and 50 nM for hesperetin-7- and 3'-sulfates respectively. LODs were 30 nM for hesperetin, 20 and 30 nM for hesperetin-7- and 3'-glucuronides respectively, and 15 and 20 nM for hesperetin-7- and 3'-sulfates respectively. The intra-day repeatability of the UHPLC-QqQ-MS method was assessed from 10 consecutive chromatographic runs using a standard solution with 2.5 µM of every standard in MeOH:0.1% (v/v) formic acid. The inter-day repeatability of the method was assessed by analyzing the same standard solution on 2 consecutive days. The relative standard deviation (RSD) for peak

area was in the range of 0.5–4.7% in the intra-day test and 1.3–3.5% in the case of the inter-day test.

Sample size and statistical analysis

To detect a 2.0 unit increase in relative FMD (the primary outcome of the present study), assuming a standard deviation of 2.0 (based on FMD reliability data), with 80% power and at the 5% significance level, a total sample size of 10 participants was required to complete a two-treatment crossover study. Data are presented as means ± SDs. Statistical analyses were conducted by using Statistical Package for the Social Sciences (SPSS, version 24, IBM Corporation, USA). Data were tested for normality by using Shapiro-Wilk test with normality defined as P>0.05. Differences in study outcomes between treatments were analyzed using a two-factor repeated-measures ANOVA, with treatment and time (baseline and week 2 within each treatment period) as within-subject factors. The main effects of treatment and time as well as the time by treatment interaction were investigated. When significant time, treatment, and/or time by treatment effects were identified, *post hoc* comparisons were carried out by using Bonferroni correction for multiple comparisons. The effect of sex on relative FMD was investigated by using a mixed ANOVA, with treatment and time as within-subject factors and sex as the between-subjects factor, including a time by treatment by sex interaction. Differences in relative FMD adjusted for BMI were analyzed by using a repeated-measures ANOVA, with treatment and time as within-subject factors and BMI as the covariate, including a time by treatment by BMI interaction. Carryover effects on relative FMD were assessed by using a mixed ANOVA, with treatment and time as within-subject factors and treatment sequence as the between-subjects factor. Missing data were not imputed and a complete case analysis was performed. The flavanone concentration in the different drinks was analyzed using an Independent-Samples T-Test. Significance was defined at $P<0.05$. Correlation analyses were conducted by using Pearson's correlation coefficient.

197 **Results**

198 Clinical characteristics of participants at screening are shown in **Table 1**. Among 16 enrolled
199 participants, 15 participants (10 female participants and 5 male participants) completed all
200 arms of the intervention. All 15 participants reported no major changes in diet and lifestyle
201 during the intervention, which was confirmed by unchanged body weight (data not shown).
202 The habitual intake of citrus fruit/juice was generally low (both less than one portion per week)
203 and the total intake of tea and coffee were 2.4 ± 1.1 cups/d (1 cup: 200-250 mL). No carryover
204 effects were observed (*P*-interaction=0.20). Resting brachial artery diameter did not differ
205 between treatments prior to the intervention (*P*=1.00; **Table 2**), however a time by treatment
206 interaction (*P*=0.017) was observed. Compared with baseline, relative FMD markedly
207 increased only following the 2-week consumption of BOJ (**Figure 2A**: time by treatment
208 interaction: *P*=0.001). Area under the shear rate curve to peak dilation (AUCpeak) (**Figure 2B**:
209 time by treatment interaction: *P*=0.56) and time to peak diameter did not change over time in
210 either treatment (Table 2). To remove the influence of changes in resting artery diameter on
211 FMD, FMD was scaled to resting artery diameter according to Atkinson (36). Analysis of the
212 scaled FMD index also revealed an increase following the 2-week consumption of BOJ only,
213 compared with baseline (*P*=0.001).
214 Plasma concentrations of estradiol in female participants did not differ during the trial periods
215 (0.33 ± 0.27 and 0.28 ± 0.25 nmol/L for pre and post BOJ consumption, respectively; 0.27 ±
216 0.16 and 0.29 ± 0.21 nmol/L for pre and post CD consumption, respectively; time by treatment
217 interaction: *P*=0.80). Moreover, the effect of the drinks on relative FMD did not differ with sex
218 (the effect of sex: *P*=0.28; *P*-interaction=0.70). Although the effect of BMI on relative FMD was
219 not significant (the effect of BMI: *P*=0.61; *P*-interaction=0.56), there was a moderate inverse
220 correlation between the BMI of participants and changes in relative FMD following 2-week
221 consumption of BOJ (*R*=-0.42, *P*=0.12). Similarly, a moderate inverse correlation between the
222 BMI of participants and changes in relative FMD was also observed following 2-week
223 consumption of CD (*R*=-0.45, *P*=0.09).

All participants complied with dietary restrictions, confirmed by low (or not detectable) urinary concentrations of hesperetin-3'-glucuronide and hesperetin-7-glucuronide at baseline (P=0.42 and P=0.39, respectively; **Figure 3**). Urinary hesperetin-3'-glucuronide and hesperetin-7-glucuronide both increased following BOJ consumption from 0.17±0.04 to 9.78±2.52 μM (P=0.007) and from 0.06±0.02 to 2.71±0.70 μM (P=0.009), respectively, but not following the CD (hesperetin-3'-glucuronide from 0.30±0.08 to 0.59±0.15 μM, and hesperetin-7-glucuronide from 0.11±0.03 to 0.16±0.04 μM). Both urinary hesperetin-3'-glucuronide (R=0.35, P=0.007) and hesperetin-7-glucuronide (R=0.32, P=0.012) were significantly correlated with relative FMD.

Blood pressure (systolic and diastolic), lipids (total cholesterol, triglycerides, and HDL-cholesterol), hsCRP, and endothelin-1 were within healthy ranges (37-40) and not affected by the interventions, except for cGMP with a significant interaction and LDL-cholesterol with a significant treatment effect (Table 2). No significant correlations were observed between these outcomes and relative FMD (data not shown).

Discussion

This study demonstrates a significant 2.01% increase in FMD following consumption of anthocyanin-rich blood orange juice as compared to a low flavonoid control drink, with a concurrent significant increase in urinary flavanone metabolites. Importantly, whilst the clinical use of FMD in the calculation of CVD risk has not been recognized, a 1% increase in FMD in large trials has been associated with a range of 8-13% reduction in CVD risk (6, 41). Furthermore, FMD is most successful to monitor effects of interventions, as seen in the current study. The increase in FMD following BOJ intake can be considered as relatively large, given that the chronic effect of flavonoids on FMD is only 0.73 % (ranging from 0.17 to 1.30) as demonstrated by a pooled analysis of flavonoid intervention trials (42). The effects of BOJ on FMD in the present study are comparable to cocoa powder (800 mg cocoa flavonoids/d for 1 week) consumed by healthy individuals (43). To our knowledge, the crossover study by Buscemi et al. (22) is the only other currently published study on BOJ consumption (500 mL/d)

that can demonstrate a significant increase in endothelial function via FMD alongside decreases in the inflammatory markers CRP, IL-6 and TNF-α after 1 week supplementation in participants with augmented CVD risk. The few other studies on BOJ supplementation were not able to show effects on biomarkers of CVD risk in participants who were overweight (500 mL/d over 28 days) (23) or only a moderate reduction in LDL cholesterol levels in participants with obesity after a 12-week supplementation with 500mL BOJ/d (24). Our current study, using a relatively high volume of 400 mL BOJ per day, did not demonstrate changes in blood pressure, lipid profiles and markers of inflammation, likely due to all values being within a healthy range. Missing data on lipid profiles and markers of inflammation might have limited impact on the interpretation of the results, since the participants were relatively young and healthy, and hence it is likely those outcomes were within a healthy range at baseline and did not change by the intervention. It cannot be excluded that spontaneous changes in the diet were made by the participants to compensate for the energy load in the drinks, as body weight remained unchanged during the study period. Since their habitual diet was low in citrus fruit/juice and not high in tea/coffee, and participants were asked to maintain their diet during the study, the effects of compensatory changes in flavonoid rich foods/drinks were considered to be negligible. This was confirmed by unchanged baseline values of urinary metabolites and endothelial function.

To our knowledge, this is the first study to report an impact of polyphenol-rich foods or drink consumption upon resting artery diameter. This indicates a small amount of remodeling of the brachial artery, which may be due to elevated levels of NO following the consumption of BOJ, evidenced by a 29% increase in plasma cGMP. Together with unchanged shear rate stimulus, it suggests a functional improvement in endothelial function probably through enhanced NO bioavailability due to anthocyanin and flavanone metabolites following BOJ consumption and hence for the same shear stimulus the reactivity of the vessel is greater. Indeed, a positive correlation was demonstrated between plasma cGMP and FMD response following a 12-week supplementation with anthocyanins in hypercholesterolemic individuals (44) with cGMP being considered as an indicator for plasma NO levels (45).

Large variations in study outcomes have been observed in previous flavonoid supplementation trials, in particular when healthy participants were recruited, making overall interpretation of supplementation effectivity difficult. Our approach was aimed at minimizing potential confounding effects of the female hormone estrogen by scheduling FMD measurements to avoid the late follicular phase. Thereby the reported improvements in endothelial function observed in the present study are likely due to the intervention and not fluctuations in hormone levels. Hence, the present study demonstrates that premenopausal women can be suitable participants for the evaluation of endothelial function under defined experimental conditions.

A further factor which may contribute to the conflicting evidence in the literature is shear rate which, if not carefully controlled, might give rise to variations in the resultant FMD values, and be mistaken as a 'functional change' after an intervention. Present improvement in endothelial function following BOJ intake was induced under unchanged shear rate conditions demonstrating a strong and significant correlation between shear rate and FMD. In support of these findings, a significant correlation has only been observed in younger (27±6 y), but not in older adults (58±4 y) (46), which may indicate the loss of endothelial functionality during aging. Likewise, time from cuff deflation to peak diameter was not affected in the present study but is positively associated with increasing age (47). Given that the present participants were young and healthy, the time to reach peak dilation following cuff deflation was relatively quick but consistent with previous research in participants of a similar age (47). Another novel finding of the current study is the differential responses of endothelial function to flavonoid-rich food consumption, depending on the BMI of participants, which to our knowledge, have not been reported in previous studies. In support of this, BMI has been highlighted as a factor impacting the responsiveness of individuals in intervention trials. Azzini et al. (24) reported a lacking/abnormal response of total and LDL cholesterol in participants with obesity compared to lower BMI female participants when given BOJ supplementation.

We demonstrate here that the increase in FMD, following a 2-week daily consumption of BOJ, is concurrent with urinary excretion of citrus flavanone metabolites hesperetin-3'- and hesperetin-7-glucuronides. Our results therefore provide compelling evidence that the *in vivo* FMD response is indeed linked to the presence of citrus flavonoids and/or their circulating metabolites. The availability of flavanones from orange juice (as a sum of small intestine and gut microbiota derived compounds) is, despite high inter-individual variation, considered high (48-50). Nevertheless, many orange juice studies are not demonstrating modulation of CVD risk biomarkers or endothelial function. Schär et al. (8) has recently shown that citrus flavonoids from juice in comparison to a hesperidin supplement are much more available to humans. However, in their acute crossover RCT neither orange juice nor hesperidin supplement were able to affect any of the outcome markers such as RH-PAT (reactive hyperemia-peripheral arterial tonometry), or CVD risk biomarkers. Although acute effects were not investigated in the present study, flavanone-rich citrus beverages have been reported to be effective at counteracting the negative impact of a double meal rich in fat on postprandial endothelial function measured by FMD at 7 h post intake (7). In comparison, the measurements in the present study were conducted approximately 12 hours following the final drink, indicating a prolonged effect of the bioactive compounds in BOJ.

Anthocyanins, as present in BOJ, are rapidly but poorly absorbed in the small intestine (51); and as a consequence, we were not able to reliably detect anthocyanin metabolites in the urine samples of participants. However, the availability and molecular effects of anthocyanins towards CVD biomarkers have been documented in a number of studies. Indeed, Speciale et al. (52) suggested that anthocyanins prevent stress-induced endothelial dysfunction. Consumption of BOJ for 3 weeks significantly increased plasma antioxidant concentrations (21) and the intake of blackcurrant juice, an abundant source of anthocyanins and other bioactives, for 6 weeks resulted in a significant increase in FMD in healthy adults (53). It has been suggested that the beneficial effects of blood orange may be mediated by the synergistic effects of its different compounds (54).

There are several limitations to the present study. First, distinct differences in the colour and taste of the two drinks made double-blinding impossible. However, the researchers who conducted the analyses of the biological samples and FMD data were blinded to which juice the participant was consuming. It is unlikely that the outcomes of the present study (such as endothelial function, lipid profile, hsCRP) were influenced by participants knowing which juice they were consuming. Second, although vitamin C concentration was not matched in the control drink, it is unlikely the observed enhancement in FMD was due to vitamin C presence. Clinical data suggest that doses of vitamin C up to 500 mg do not alter endothelial function, both acutely and chronically (55); and vitamin C concentration in the BOJ ingested in the present study was only 168 mg per day. In addition, given the short half-life of vitamin C (approximately 30 min), it seems unlikely that vitamin C exerted any effect on the markers determined after a 12 h overnight fast. Sex difference analysis was of course hampered by the small sample size. Although Bonferroni corrections were used, there is always a chance of making a Type I error in any study testing multiple secondary outcomes.

In conclusion, we observed favourable changes in resting arterial tone and endothelial function in healthy Caucasian men and premenopausal women with overweight or obesity following the consumption of blood orange juice. Further studies are required to better understand the role and potential interactions of individual flavonoids and their metabolites in BOJ and their contribution to reducing CVD risk. The differential effects on FMD according to BMI warrant further confirmation in larger cohorts. In addition, future RCTs on specific participant groups (based on age, sex, ethnicity/genotype, BMI, CVD risk) are needed to investigate effects of polyphenol-rich products following long term consumption.

Acknowledgements

We thank Saïd Ibeggazene for his support with FMD practice and Fraser Chadwick for his help with venepuncture.

The authors' contributions were as follows: LL, KMB, CB designed the study; LL conducted the human study and statistical analyses; GKL trained LL in FMD and provided venepuncture

support; GKL and KMB scaled FMD data and conducted statistical analyses on scaled FMD index; JAMB, FV, FTB analyzed urinary flavanone metabolites; LL drafted the manuscript which was finally edited by GKL, CB and KMB. All authors have read and approved the final manuscript.

References

1. Cassidy A, Bertoia M, Chiuve S, Flint A, Forman J, Rimm EB. Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. Am J Clin Nutr 2016;104:587-94.
2. Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, Hu FB, van Dam RM. Selected Dietary Flavonoids Are Associated with Markers of Inflammation and Endothelial Dysfunction in U.S. Women. J Nutr 2011;141:618-25.
3. Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Laganà G, Daglia M, Meneghini S, Nabavi SM. Flavanones: citrus phytochemical with health-promoting properties. BioFactors 2017;43:495-506.
4. Testai L, Calderone V. Nutraceutical value of citrus flavanones and their implications in cardiovascular disease. Nutrients 2017;9:502.
5. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiol 2009;196:193-222.
6. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging 2010;26:631-40.
7. Rendeiro C, Dong H, Saunders C, Harkness L, Blaze M, Hou Y, Belanger RL, Corona G, Lovegrove JA, Spencer JP. Flavanone-rich citrus beverages counteract the transient decline in postprandial endothelial function in humans: a randomised, controlled, double-masked, cross-over intervention study. Br J Nutr 2016;116:1999-2010.
8. Schär MY, Curtis PJ, Hazim S, Ostertag LM, Kay CD, Potter JF, Cassidy A. Orange juice–derived flavanone and phenolic metabolites do not acutely affect cardiovascular risk biomarkers: a randomized, placebo-controlled, crossover trial in men at moderate risk of cardiovascular disease. Am J Clin Nutr 2015;101:931-8.

9. Morand C, Dubray C, Milenkovic D, Lioger D, Martin JF, Scalbert A, Mazur A. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J Clin Nutr 2011;93:73-80.

10. Rees A, Dodd G, Spencer J. The Effects of Flavonoids on Cardiovascular Health: A Review of Human Intervention Trials and Implications for Cerebrovascular Function. Nutrients 2018;10:1852.

11. Cassidy A, Minihane A-M. The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr 2016;105:10-22.

12. Adkisson EJ, Casey DP, Beck DT, Gurovich AN, Martin JS, Braith RW. Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. Exp Biol Med 2010;235:111-8.

13. Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, Komesaroff PA. Variations in endothelial function and arterial compliance during the menstrual cycle. J Clin Endocrinol Metab 2001;86:5389-95.

14. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011;300:H2-H12.

15. Manach C, Milenkovic D, Wiele T, Rodriguez-Mateos A, Roos B, Garcia-Conesa MT, Landberg R, Gibney ER, Heinonen M, Tomás-Barberán F. Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction. Mol Nutr Food Res 2017;61:1600557.

16. Day AJ, Cañada FJ, Díaz JC, Kroon PA, Mclauchlan R, Faulds CB, Plumb GW, Morgan MR, Williamson G. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett 2000;468:166-70.

17. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov 2008;7:156-67.

18. Chang JS, Hsiao J-R, Chen C-H. ALDH2 polymorphism and alcohol-related cancers in Asians: a public health perspective. J Biomed Sci 2017;24:19.

19. Brennan P, Lewis S, Hashibe M, Bell DA, Boffetta P, Bouchardy C, Caporaso N, Chen C, Coutelle C, Diehl SR. Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer: a HuGE review. Am J Epidemiol 2004;159:1-16.

20. Rapisarda P, Carollo G, Fallico B, Tomaselli F, Maccarone E. Hydroxycinnamic acids as markers of Italian blood orange juices. J Agric Food Chem 1998;46:464-70.

21. Riso P, Visioli F, Gardana C, Grande S, Brusamolino A, Galvano F, Galvano G, Porrini M. Effects of Blood Orange Juice Intake on Antioxidant Bioavailability and on Different Markers Related to Oxidative Stress. J Agric Food Chem 2005;53:941-7.

22. Buscemi S, Rosafio G, Arcoleo G, Mattina A, Canino B, Montana M, Verga S, Rini G. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. Am J Clin Nutr 2012;95:1089-95.

23. Hollands WJ, Armah CN, Doleman JF, Perez-Moral N, Winterbone MS, Kroon PA. 4-Week consumption of anthocyanin-rich blood orange juice does not affect LDL-cholesterol or other biomarkers of CVD risk and glycaemia compared with standard orange juice: a randomised controlled trial. Br J Nutr 2018;119:415-21.

24. Azzini E, Venneria E, Ciarapica D, Foddai M, Intorre F, Zaccaria M, Maiani F, Palomba L, Barnaba L, Tubili C. Effect of Red Orange Juice Consumption on Body Composition and Nutritional Status in Overweight/Obese Female: A Pilot Study. Oxid Med Cell Longev 2017;2017.

25. Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, Hennekens CH. Weight, weight change, and coronary heart disease in women: risk within the 'normal' weight range. JAMA 1995;273:461-5.

26. Woo KS, Chook P, Chung WY, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation 2004;109:1981-6.

27. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, Mattioli PL. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress. Diabetes 2001;50:159-65.

28. Sweidan AMA. Bioavailability of citrus flavanones and their effect on cardiovascular health biomarkers. University of Leeds, 2015.

29. Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int 2005;88:1269-78.

30. Øbro J, Harholt J, Scheller HV, Orfila C. Rhamnogalacturonan I in Solanum tuberosum tubers contains complex arabinogalactan structures. Phytochemistry 2004;65:1429-38.

31. Harris E, Rakobowchuk M, Birch KM. Interval exercise increases angiogenic cell function in postmenopausal women. BMJ Open Sport Exerc Med 2017;3:e000248.

32. Harris E, Rakobowchuk M, Birch KM. Sprint interval and sprint continuous training increases circulating CD34+ cells and cardio-respiratory fitness in young healthy women. PloS One 2014;9:e108720.

33. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. Hypertension 2010;55:1075-85.

34. Rakobowchuk M, Harris E, Taylor A, Baliga V, Cubbon RM, Rossiter HB, Birch KM. Heavy and moderate interval exercise training alters low-flow-mediated constriction but does not increase circulating progenitor cells in healthy humans. Exp Physiol 2012;97:375-85.

35. Rangel-Huerta OD, Aguilera CM, Martin MV, Soto MJ, Rico MC, Vallejo F, Tomas-Barberan F, Perez-de-la-Cruz AJ, Gil A, Mesa MD. Normal or High Polyphenol Concentration in Orange Juice Affects Antioxidant Activity, Blood Pressure, and Body Weight in Obese or Overweight Adults. J Nutr 2015;145:1808-16.

36. Atkinson G. Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas 2014;35(9):1825.

37. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, De Simone G, Dominiczak A. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). Eur Heart J 2018;39:3021-104.

38. Program NCE. Expert Panel on Detection, Evaluation, and treatment of high blood cholesterol in Adults (Adult treatment Panel III). Third report of the National cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and treatment of high blood cholesterol in Adults (Adult treatment Panel III) final report. Circulation 2002;106:3143-421.

39. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon III RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499-511.

40. Abdel-Sayed S, Nussberger J, Aubert J-F, Gohlke P, Brunner HR, Brakch N. Measurement of plasma endothelin-1 in experimental hypertension and in healthy subjects*. Am J Hypertens 2003;16:515-21.

41. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol 2013;168:344-51.

42. Kay CD, Hooper L, Kroon PA, Rimm EB, Cassidy A. Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. Mol Nutr Food Res 2012;56:1605-16.

43. Grassi D, Desideri G, Necozione S, Di Giosia P, Barnabei R, Allegaert L, Bernaert H, Ferri C. Cocoa consumption dose-dependently improves flow-mediated dilation and arterial stiffness decreasing blood pressure in healthy individuals. J Hypertens 2015;33:294-303.

44. Zhu Y, Xia M, Yang Y, Liu F, Li Z, Hao Y, Mi M, Jin T, Ling W. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. Clin Chem 2011;57:1524-33.

45. Minamino T, Kitakaze M, Sato H, Asanuma H, Funaya H, Koretsune Y, Hori M. Plasma levels of nitrite/nitrate and platelet cGMP levels are decreased in patients with atrial fibrillation. Arterioscler Thromb Vasc Biol 1997;17:3191-5.

46. Thijssen DH, Bullens LM, van Bemmel MM, Dawson EA, Hopkins N, Tinken TM, Black MA, Hopman MT, Cable NT, Green DJ. Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. Am J Physiol Heart Circ Physiol 2009;296:H57-H64.

47. Black MA, Cable NT, Thijssen DH, Green DJ. Importance of measuring the time course of flow-mediated dilatation in humans. Hypertension 2008;51:203-10.

48. Pereira-Caro G, Borges G, Van Der Hooft J, Clifford MN, Del Rio D, Lean ME, Roberts SA, Kellerhals MB, Crozier A. Orange juice (poly) phenols are highly bioavailable in humans. Am J Clin Nutr 2014;100:1378-84.

49. Brett GM, Hollands W, Needs PW, Teucher B, Dainty JR, Davis BD, Brodbelt JS, Kroon PA. Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. Br J Nutr 2008;101:664-75.

50. Vallejo F, Larrosa M, Escudero E, Zafrilla MP, Cerdá B, Boza J, García-Conesa MT, Espín JC, Tomás-Barberán FA. Concentration and Solubility of Flavanones in Orange Beverages Affect Their Bioavailability in Humans. J Agric Food Chem 2010;58:6516-24.

51. Vitaglione P, Donnarumma G, Napolitano A, Galvano F, Gallo A, Scalfi L, Fogliano V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. J Nutr 2007;137:2043-8.

52. Speciale A, Cimino F, Saija A, Canali R, Virgili F. Bioavailability and molecular activities of anthocyanins as modulators of endothelial function. Genes Nutr 2014;9:404.

53. Khan F, Ray S, Craigie AM, Kennedy G, Hill A, Barton KL, Broughton J, Belch JJF. Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: A randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice. Free Radic Biol Med 2014;72:232-7.

54. Grosso G, Galvano F, Mistretta A, Marventano S, Nolfo F, Calabrese G, Buscemi S, Drago F, Veronesi U, Scuderi A. Red orange: experimental models and epidemiological evidence of its benefits on human health. Oxid Med Cell Longev 2013;2013.

55. Ashor AW, Siervo M, Lara J, Oggioni C, Afshar S, Mathers JC. Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials. Br J Nutr 2015;113:1182-94.

Tables

TABLE 1

Clinical characteristics of study participants at baseline [1]

	Values
Age (y)	28.7 ± 6.5 (20-45)
BMI (kg/m^2)	28.3 ± 3.1 (25.5-36.5)
SBP (mmHg)	110.0 ± 12.9 (91.0-128.7)
DBP (mmHg)	71.9 ± 9.5 (59.3-92.3)
Total cholesterol (mmol/L)	4.7 ± 0.6 (4.0-5.8)
HDL-cholesterol (mmol/L)	1.5 ± 0.5 (0.8-2.1)
LDL-cholesterol (mmol/L)	2.6 ± 0.4 (2.1-3.2)
Triglycerides (mmol/L)	1.3 ± 0.6 (0.5-2.8)
hsCRP (mg/L)	0.9 ± 0.9 (0.2-3.4)

[1]Values are mean ± SD (range), n=15 except for lipids and hsCRP, n=10. DBP, diastolic blood pressure; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

Table 2

Endothelial function, blood pressure, and circulating inflammatory markers, lipids, endothelin 1, and cGMP in healthy adults with overweight and obesity at baseline and following 2-week consumption of blood orange juice or control drink in a random sequence [1]

	Blood orange juice		Control drink				
	Basal	2 wk	Basal	2 wk	*P*-treatment[2]	*P*-time[3]	*P*-interaction[4]
Resting diameter (mm)	3.62 ± 0.56	3.64 ± 0.54	3.63 ± 0.57	3.62 ± 0.56	0.77	0.36	0.017
AUCpeak (1000 a.u.)	55.4 ± 19.1	57.3 ± 22.3	57.4± 27.4	56.6 ± 21.0	0.25	0.91	0.56
Absolute FMD (mm)	0.29 ± 0.08[b]	0.36 ± 0.09[a]	0.29 ± 0.07[b]	0.27 ± 0.07[b]	0.001	0.06	0.001
Relative FMD (%)	8.15 ± 2.92[b]	10.2 ± 3.31[a]	8.11 ± 2.52[b]	7.77 ± 2.43[b]	0.001	0.06	0.001
Scaled FMD index	1.23±0.03[b]	1.25±0.03[a]	1.23±0.02[b]	1.22±0.02[b]	0.001	0.06	<0.001
Time to peak diameter (s)	47.6 ± 13.6	47.5 ± 14.8	47.5 ± 17.1	46.5 ± 17.7	0.88	0.82	0.83
SBP (mmHg)	108 ± 12	108 ± 11	108 ± 11	108 ± 11	0.97	0.70	0.97
DBP (mmHg)	71 ± 8	69 ± 7	72 ± 9	70 ± 8	0.61	0.06	0.87
hsCRP (mg/L)	0.59 ± 0.29	0.58 ± 0.35	0.87 ± 0.82	1.14 ± 1.47	0.15	0.64	0.63
Total cholesterol (mmol/L)	4.63 ± 0.60	4.68 ± 0.56	4.55 ± 0.57	4.54 ± 0.77	0.24	0.82	0.81
Triglycerides (mmol/L)	1.23 ± 0.77	1.28 ± 0.61	1.38 ± 0.68	1.28 ± 0.61	0.58	0.37	0.57
LDL-cholesterol (mmol/L)	2.62 ± 0.39	2.60 ± 0.23	2.39 ± 0.46	2.46 ± 0.46	0.005	0.78	0.74
HDL-cholesterol (mmol/L)	1.53 ± 0.49	1.57 ± 0.57	1.59 ± 0.57	1.50 ± 0.49	0.82	0.61	0.27
Endothelin 1 (pg/mL)	1.08 ± 0.18	1.05 ± 0.27	1.15 ± 0.22	1.09 ± 0.29	0.51	0.40	0.83
cGMP (pmol/mL)	61.2 ± 19.0	78.9 ± 29.1	72.4 ± 15.0	65.9 ± 16.5	0.84	0.37	0.043

[1]Values are mean ± SD, n=15 except for lipids, endothelin 1, cGMP and hsCRP, n=10. Values with different superscript letters differ (P<0.05). AUCpeak, area under the shear rate curve to peak dilation; cGMP, cyclic guanosine monophosphate; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

[2]Indicates the main effect of treatment, P<0.05.

[3]Indicates the main effect of time, P<0.05.

[4]Indicates the time by treatment interaction, P<0.05.

Legends for figures

Figure 1

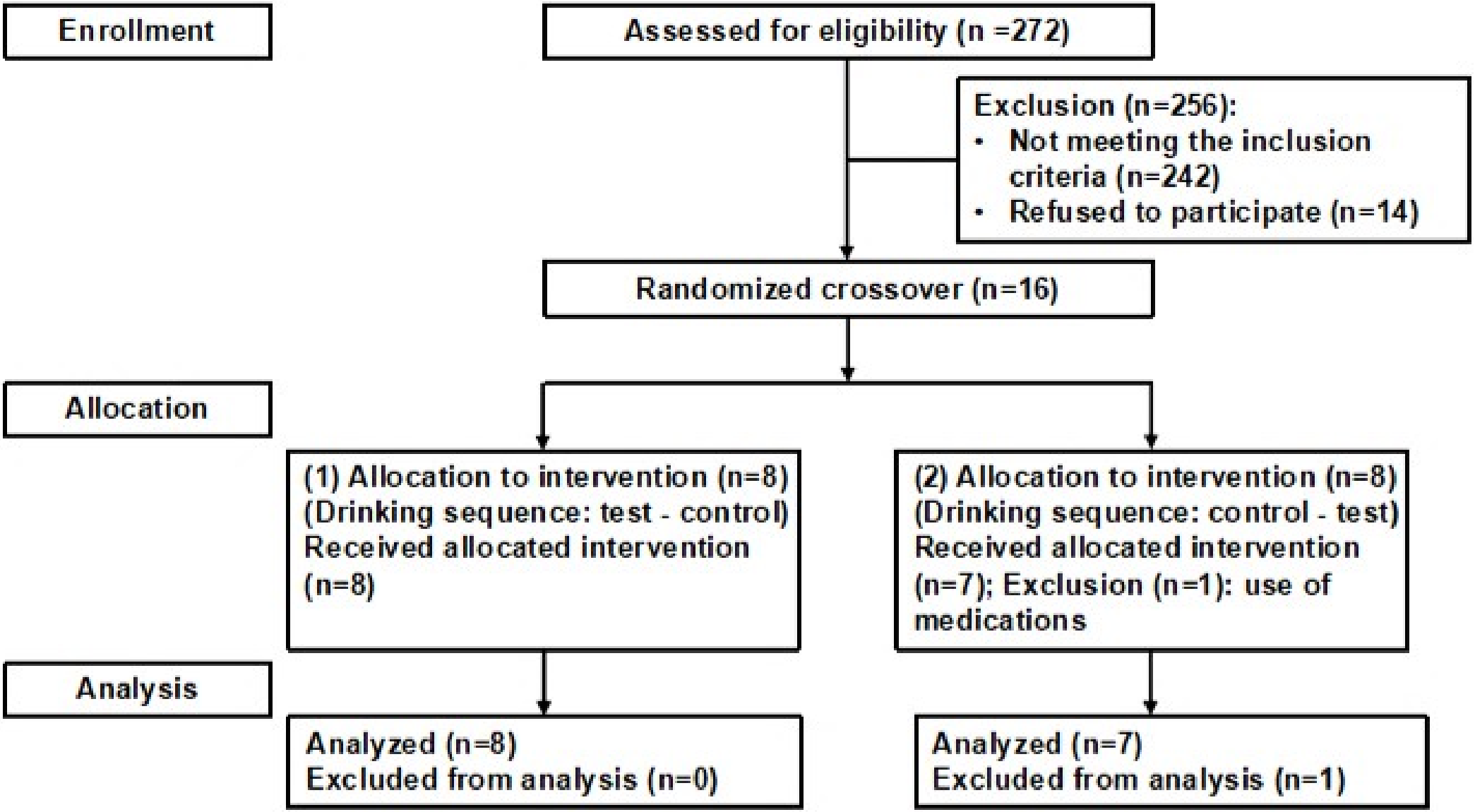
Participant flow diagram. Simple randomization was used to determine the different groups (starting with test drink or control drink). Block randomization was conducted to randomly allocate participants into groups to ensure an equal number in each group.

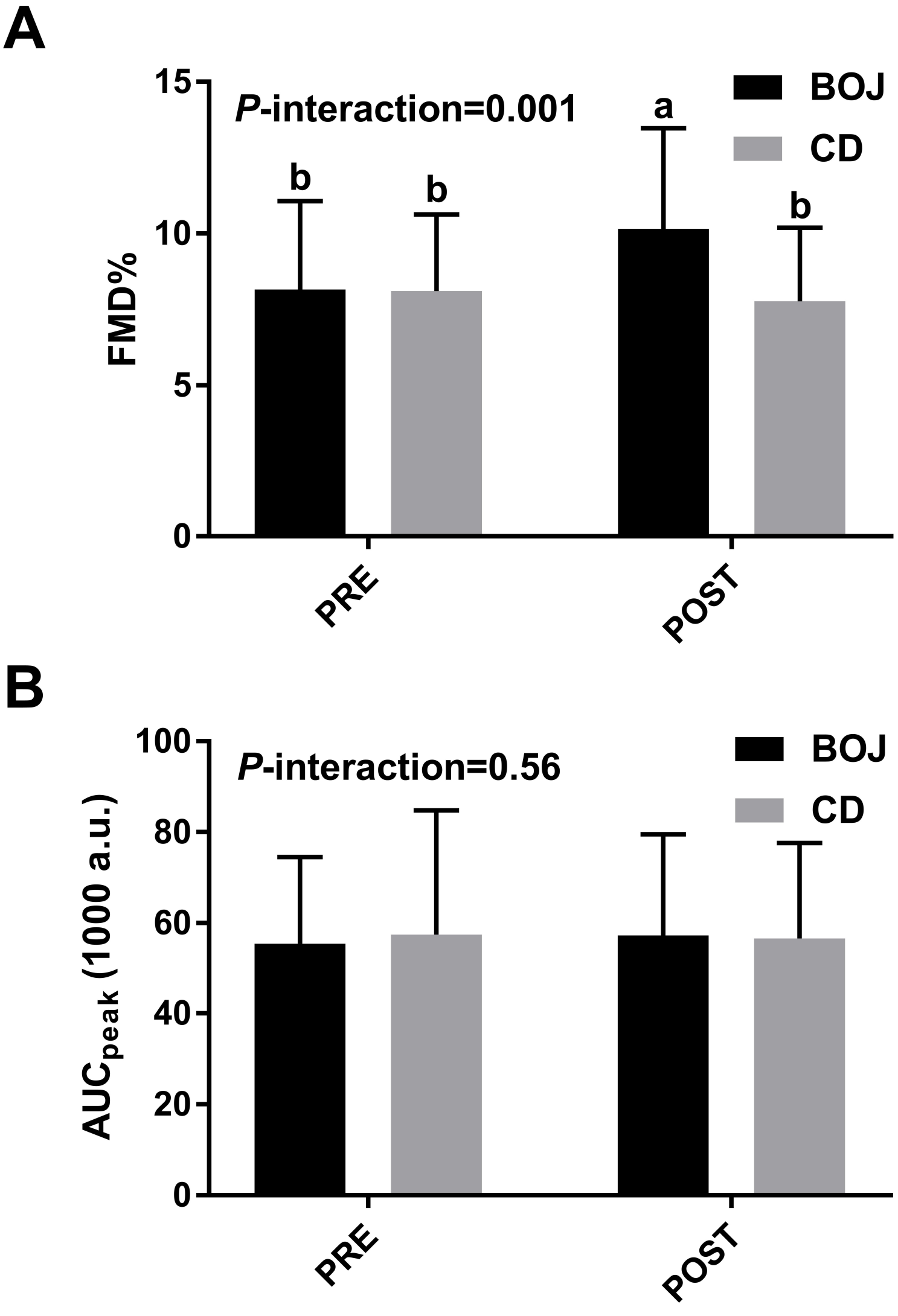
Figure 2

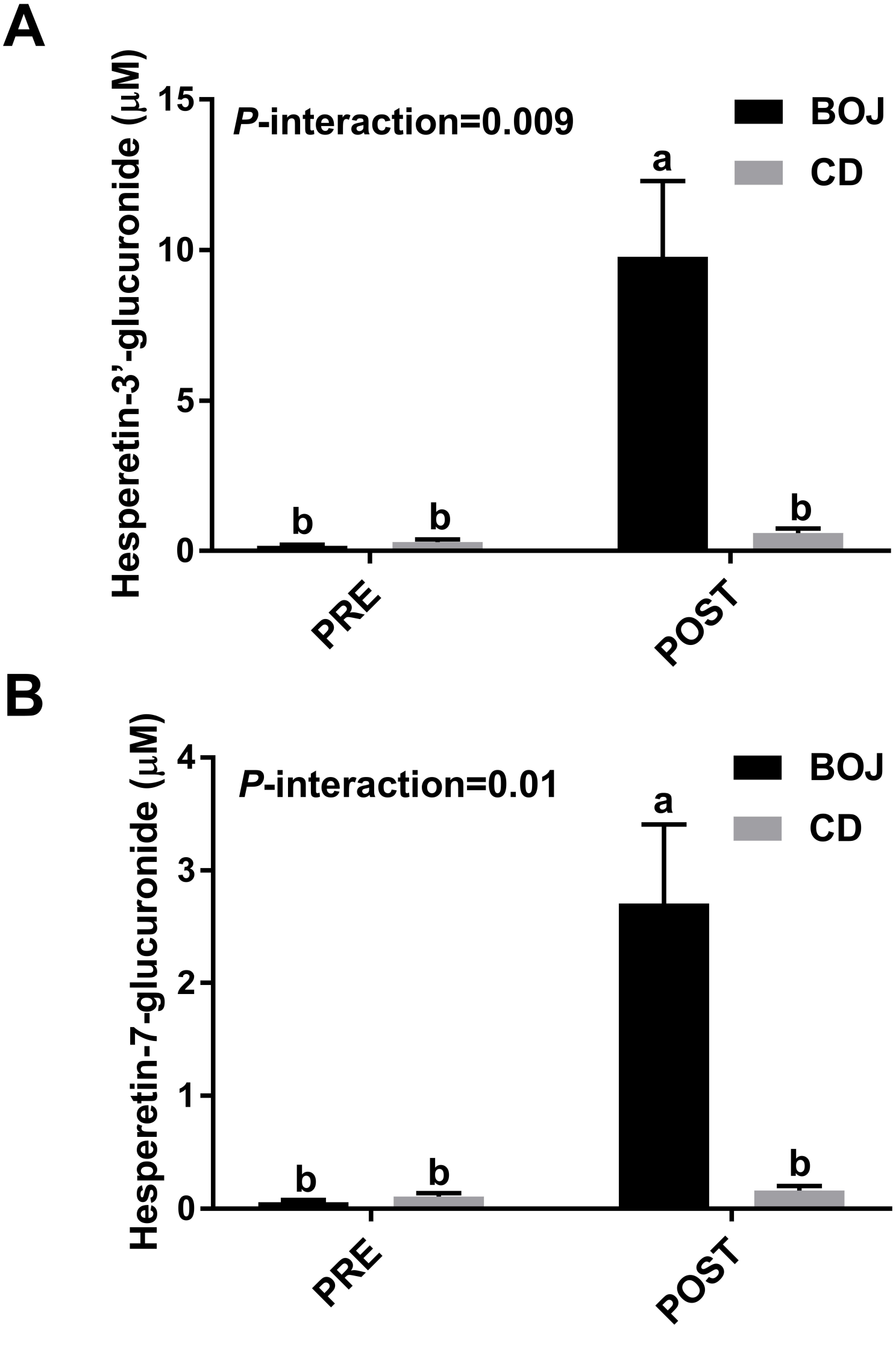
Endothelial function in healthy adults with overweight and obesity prior to and following 2-week consumption of blood orange juice or control drink (2 x 200 mL/d) in randomized order. (A) Relative FMD, (B) AUCpeak. Data are mean ± SD, n=15. Labeled means without a common letter differ, $P<0.05$. AUCpeak, area under the shear rate curve to peak dilation; BOJ, blood orange juice; CD, control drink; FMD, flow-mediated dilation.

Figure 3

Urinary flavanone metabolites hesperetin-3'-glucuronide (A) and hesperetin-7-glucuronide (B) in healthy adults with overweight and obesity prior to and following consumption of blood orange juice or control drink (2 x 200 mL/d) in randomized order. Data are mean ± SD, n=15. Labeled means without a common letter differ, $P<0.05$. BOJ, blood orange juice; CD, control drink.


Enrollment
Assessed for eligibility (n =272)
Exclusion (n=256):
• Not meeting the inclusion criteria (n=242)
• Refused to participate (n=14)
Randomized crossover (n=16)
Allocation
(1) Allocation to intervention (n=8)
(Drinking sequence: test - control)
Received allocated intervention (n=8)
(2) Allocation to intervention (n=8)
(Drinking sequence: control - test)
Received allocated intervention (n=7); Exclusion (n=1): use of medications
Analysis
Analyzed (n=8)
Excluded from analysis (n=0)
Analyzed (n=7)
Excluded from analysis (n=1)


A
15
10
5
0
FMD%
P-interaction=0.001
BOJ
CD
b
b
a
b
PRE
POST
B
100
80
60
40
20
0
AUCpeak (1000 a.u.)
P-interaction=0.56
BOJ
CD
PRE
POST


A
Hesperetin-3'-glucuronide (μM)
15
10
5
0
P-interaction=0.009
BOJ
CD
a
b
b
b
PRE
POST
B
Hesperetin-7-glucuronide (μM)
4
3
2
1
0
P-interaction=0.01
BOJ
CD
a
b
b
b
PRE
POST