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Anthocyanin-rich plum juice reduces ambulatory blood pressure but not acute cognitive function in younger and older adults: a pilot crossover dose-timing study

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ABSTRACT

Consumption of anthocyanins from fruit sources may exert protection against hypertension and improve cognition. However, the effect of dose timing in studies is rarely considered. We hypothesized that timed-dose consumption of juice from an anthocyanin-rich Japanese plum variety (Queen Garnet plum, QGP) will have acute and dose-timing effects on cardiovascular responses, cognition, and urinary anthocyanin excretion profiles. Our study objective was to investigate the impact of plum juice on these health parameters. Twelve older (65+ years) and 12 younger (18-45 years) adults participated in an acute crossover study. Participants received, randomly, either 1 × 300 mL or 3 × 100 mL plum juice over 3 hours on 2 different occasions with a 2-week washout period. A battery of cognitive tasks was administered at 0 and 6 hours on each study day. Blood pressure (BP) and urinary anthocyanin/metabolite excretion profiles were measured over 24 hours. Area under the curve for BP was calculated (0-6 hours). A significant reduction in BP and cardiovascular responses was observed in both age groups which was more obvious in the older age group on the single dose for systolic BP, diastolic BP, mean arterial pressure, and heart rate (*P* values = .035, .028, .017, and .006, respectively). No significant difference was observed between dose-timing regimens for either age group. There was no observed effect on cognition. Native QGP anthocyanins, as well as methylated/glucuronidated metabolites, were detected in urine with no significant differences between age groups or dose timing. High-anthocyanin plum juice significantly reduced BP, but dose timing did not appear to be a significant factor in the potential acute BP-lowering effect of QGP juice.

Abbreviations: ABPM, ambulatory blood pressure monitor; ANOVA, analysis of variance; AUC, area under curve; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; HR, heart rate; LDL, low-density lipoprotein; MAP, mean arterial pressure; MAO, monoamine oxidase; QGP, Queen Garnet plum; QGPJ, Queen Garnet plum juice; SBP, systolic blood pressure; UOW, University of Wollongong.

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1. Introduction

Elevated and high blood pressure (BP) is a major public health concern and a significant risk factor for cardiovascular diseases (CVDs). According to the World Health Organization, the prevalence of high BP in adults 18 years and older was around 22% in 2014. This accounted for about 9.4 million deaths or 7% of all deaths [1]. In Australia, between 2011 and 2012, almost one-third (31.6%) of all adults were diagnosed with hypertension, which was more prevalent at older ages, with almost 9 in 10 (87.7%) people 85 years and older being hypertensive [2]. In global strategies to address noncommunicable diseases including hypertension, the significant role of modifiable dietary risk factors, including an increased intake of fruits and vegetables, is acknowledged [3]. Small (2-5 mm Hg) but steady decreases in mean BP have been shown to significantly decrease the incidence of cardiovascular events [4]. Given the magnitude of hypertension and its contribution toward the burden of CVD, cost-effective strategies including dietary intervention are needed for its prevention and management. Plant-based foods are integral to a healthy human diet, and a plant-rich diet is associated with the prevention of a vast array of diseases [5]. Bioactive compounds of interest include polyphenols, which are found mainly in plant-based foods and have antioxidant properties. More than 8000 different polyphenols have been identified in nature within 4 different categories (flavonoids, phenolic acids, lignans, and stilbenes). Over the past decade, there has been increased research into flavonoids, notably, anthocyanins, for their beneficial health effects [6].

Anthocyanins, the largest subclass of flavonoids, comprise a group of water-soluble phytochemicals known to be responsible for the deep rich red to blue-purple colors in fruits and vegetables [7]. There is some evidence from epidemiological studies that suggests that a higher consumption of anthocyanin-rich foods is associated with a reduced risk for CVD [8,9]. However, intervention studies do not always support these findings [10]. In the case of BP, plausible mechanisms from experimental studies include their effects on vascular blood flow and flow-mediated dilation [11,12].

It has been hypothesized that anthocyanins may exert protective effects on cognition, including memory and executive processing, either through a direct effect on brain function or indirectly by reducing BP [13-15]. One of the main pathways linking BP to cognitive degeneration is the decline in vascular reserve capacity which is associated with impaired neurovascular coupling [16]. Despite evidence from epidemiological and intervention studies indicating that anthocyanin intake is linked with improved cognition [15,17] and a slower cognitive decline [18], the mechanisms by which anthocyanins may exert acute effects on brain function remain unclear and evidence is inconsistent. A crossover study by Caldwell et al (2016) [19] found that high-anthocyanin cherry juice consumption did not result in any significant acute effects on a battery of cognitive tests in either younger or older adults. Contrary to this, Watson et al (2015) [20] observed a cognitive benefit of acute blackcurrant supplementation in healthy younger adults possibly explained by an association between monoamine oxidase (MAO) inhibition and improved attention. There is a possibility that the inhibition of MAO has positive effects on monoaminergic neurotransmission during cognitive performance [21]. This is as a result of monoamine levels, particularly for dopamine, being shown to increase during cognitive tasks (which assess working memory and attention) with a positive correlation with task performance [21]. An acute effect on cognition by fruit anthocyanin supplementation has also been observed in children [17,22].

Inadequate understanding of the uptake, metabolism distribution, and excretion of anthocyanins has limited the design of clinical trials that investigate their effect on health outcomes. The body of evidence on the protective effects of flavonoid-rich foods against CVD is based mainly on epidemiological studies; thus, evidence remains inconclusive, and acute effects have not been well defined. Systematic reviews of available experimental studies [23,24] have highlighted an absence of knowledge regarding a "threshold dose" or appropriate "dose timing" required to induce physiological protective effects. This is because the impact of anthocyanin dose has not been studied extensively in humans and different experiments have used varied preparations, for example, juice, puree, and whole fruit. Consequently, studies administer unfeasibly large doses of anthocyanin-rich foods to elucidate a physiological response, and the selection of dose timings is often unsubstantiated [25-27]. Although splitting a large daily dose of anthocyanin-rich food into 3 or more servings per day may reflect a more feasibly tolerated serve, there are often no justification as to the reason each dose was selected and no consideration given to the physiological effects. Although results have mostly been in agreement, evidence shows that, beyond a point, the bioavailability of anthocyanins decreases with increasing dose [28]. For cyanidin-based anthocyanins, the maximum absorption has been reported to be about 350 μmol or less. This is also believed to differ according to the structure of different anthocyanins and to the attached sugar moiety and because of wide interindividual variation in metabolism which limits translation of research findings into dietary messages [29]. Taking these factors into consideration, there is a need to better understand the acute effects of anthocyanins provided from different foods and beverages to identify any consistent potential health benefits.

The increased interest in anthocyanin-based research has translated into agricultural responses, as the demand for fruits with superior health benefit grows. An example is the Queen Garnet plum (QGP), which is a variety of the popular Japanese plum *Prunus salicina Lindl* that was bred by the Queensland Government to be very high in anthocyanins, providing up to 277 mg/100 g of fruit [30] under "optimal" environmental and harvest conditions. This is more than twice the anthocyanin content of regular plums that ranges from 5 to 173 mg/100 g across harvest years [30]. Previous work from our group has determined the acute effect of anthocyanins provided from a different fruit source (cherries) [15,31]. Learnings from those studies underpin the improved methodologies used in the current study, particularly with regard to more robust assessment of 24-hour BP and measurement of urinary anthocyanin metabolites. Furthermore, the QGP juice (QGPJ) has a completely different anthocyanin and nonanthocyanin polyphenol profile compared with the Australian cherry juice vehicle used previously [15,31], which

may influence its synergistic/antagonist effects on biological activities. Our previous acute trial found that plasma levels of anthocyanin-related metabolites were significantly lower for older adults, but not for younger adults, who consumed cherry juice over 3 smaller servings (3 × 100 mL) compared with consumption of 300 mL at a single time point. This finding warrants further research consideration.

As a follow-up, this study hypothesized that the consumption of high-anthocyanin QGPJ will:

1. Have an acute beneficial effect on various domains of cognitive functioning and BP,
2. Be found to be bioavailable through the presence of anthocyanin metabolites excreted in the urine over a 24-hour period, and
3. Show differences in the absorption rate and metabolism of anthocyanins between young and older adults.

From the above hypotheses, the primary aim of this study was to determine the dose-timing response on acute ambulatory BP and cognitive function following consumption of 300 mL QGPJ, provided as either a single dose or three 100-mL quantities over 3 hours, in young and older adults. The secondary outcome was to determine the bioavailability of QGP anthocyanins, as assessed by urinary excretion over a 24-hour period, and to assess any significant differences in the anthocyanin/metabolite profiles between young and older adults.

2. Methods and materials

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Wollongong (UOW) Human Research Ethics Committee, New South Wales, Australia (HE16/278). Written informed consent was obtained from all participants.

2.1. Study design

The study was a pilot crossover bioequivalence/noninferiority study to assess the acute impact of differing dose timings of high-anthocyanin plum juice consumption on acute cognition and BP over 24 hours. This study was considered a bioequivalence/noninferiority design because of the dosage for the 2 crossover arms being the same (a single 300 mL and 3 × 100 mL over 3 hours) with the aim to determine whether three 100 mL of plum juice taken over 3 hours would have a different pattern of effect on BP in comparison to a single 300-mL dose. Results from this pilot study will inform the methodology as well as sample size calculation for a future crossover randomized clinical trial. This is in line with the assumptions of bioequivalence studies whereby the future trial will be designed as a crossover study [32-34].

2.2. Participants

All participants were recruited from the UOW and the surrounding Wollongong areas through poster advertising. Potential participants had the opportunity to discuss the study over the phone prior to clinic visits and were screened to determine eligibility.

Twenty-four participants were recruited, including 12 younger (18-45 years) and 12 older (65+ years) adults. The sample size was determined according to recommendations for planning a pilot study that investigates bioavailability and bioequivalence of components within food [32-34].

Recruited eligible participants were randomized to a dose-timing allocation and cognitive assessment order by a computer-generated block randomization by an independent statistician. Participants attended two 6-hour clinic visits at the Illawarra Health and Medical Research Institute at the UOW, New South Wales, Australia, between June and September 2015 with at least a 2-week washout period between clinic visits.

2.3. Exclusion criteria

Exclusion criteria included self-reported uncontrolled hypertension, any unstable physical or mental health condition, inability to provide informed consent, consumption of specific daily health supplements, and inability to communicate in the English language.

2.4. Data collection

On the first study day, a questionnaire was administered to determine participants' sociodemographic characteristics. The International Physical Activity Questionnaire validated by Hagströmer et al [35] was used to determine habitual level of physical activity, and BP measurements were taken using an ambulatory blood pressure monitor (ABPM) (Model 90207; Spacelabs Medical Inc, Issaquah, WA, USA).

2.4.1. Dietary instruction and intervention meals

The QGPJ was used as the vehicle to provide a specific and consistent anthocyanin dose to study participants. The plum juice was produced from a single seasonal batch; was processed to juice by research partners at the Department of Agriculture and Fisheries, Queensland Government; and was batch frozen at –20°C until usage [36].

Prior to each study day, participants were advised to avoid consumption of purple/red fruits and vegetables including wine, juices, jams, and smoothies in the 24-hour periods immediately before and after interview day. Verbal compliance to this was received prior to the study. On each study day, participants arrived between 08:00 and 09:30 hours at the clinic facility following a 12-hour fast. A spot urine sample was collected and a battery of cognitive tests was administered by 2 interviewers who had been trained by a senior psychologist (SR). Thereafter, a standardized breakfast (Weet-Bix, milk, and sugar) that was low in flavonoids was provided. QGPJ was provided with breakfast in random order as either (1) a single dose of 300 mL (369 mg total anthocyanins) or (2) 3 × 100-mL servings (123 mg total anthocyanins/serving) of the same plum juice at 0, 1, and 3 hours. A standardized snack (ham and cheese sandwich) was provided at 4 hours, and two (250 mL) bottles of water was provided for the 6-hour duration spent in the study facility to be consumed ad libitum.

2.4.2. Ambulatory 24-hour BP and anthropometric measurements

BP was measured using ABPMs for improved monitoring over 24 hours in comparison to standard digital BP monitors used in similar studies over a 6-hour period [15,31].

Upon arrival at the testing facility, participants were fitted with an ABPM (Model 90207; Spacelabs Medical Inc, Issaquah, WA, USA). The ABPM took BP measurements over the next 24

hours: every 15 minutes while at the testing facility (first 6 hours) and thereafter once per hour while at home. The ABPM uses an oscillometric method for the detection of systolic (SBP) and diastolic blood pressure (DBP) and has been shown to be more accurate than casual or in-office BP measurements [37]. Participants were encouraged to go about their usual daily activities but were advised to stand still and relax their arm whenever the monitor recorded measurements, that is, cuff inflation and deflation. After 24 hours, the monitor was removed and collected from participants' homes, and data were downloaded from the monitor for analysis.

Height (in meters) and weight (in kilograms) were measured using a stadiometer (Seca, Hamburg, Germany) and an electronic scale (Omron HN286 Digital Personal Body Weight Scale; Omron, Silverwater, New South Wales, Australia), respectively, to 2 decimal places, and body mass index (weight/[height^2]) was calculated.

2.4.3. *Cognitive tasks*

Five short cognitive interviewer-administered tests [38,39] were administered by trained investigators at baseline and 6 hours on both testing occasions to determine any acute changes in cognition.

The total duration of the battery of tasks was approximately 30 minutes. To control for crossover effects, there were 4 versions of the cognitive battery so that each participant had a different version at baseline and 6 hours and also after crossover period.

The Trail Making Test [40] required participants to alternate selective responses between 2 types of stimuli in the one task. The difference in the number of seconds required to complete the task was compared to a nonswitching version. This task assesses higher executive function.

In the Rey Auditory Verbal Learning Test [41], participants learn and recall a list of words over 5 trials, and each correct word that is identified is associated with a score. This task assesses verbal learning and memory.

The Pattern and Letter Comparison task [42] requires participants to compare strings of patterns or letters to determine if it is the same or different. They are required to complete as many examples as possible in 30 seconds, and scores are tallied. This task assesses speed of processing.

The Reaction Time task [43] involves display of a left or right arrow-shaped stimulus on the computer screen, and participants are required to press the corresponding mouse button (left or right). Outcome variables are proportion of correct responses and latency (response speed). This task assesses general alertness and speed of processing.

The Stroop task [44] provides participants with a sheet on which the words *purple*, *green*, *yellow*, *red*, and *blue* are printed (50 in total). Each word is shown in either congruent or incongruent ink colors (eg, the word "blue" printed in red). Participants are instructed to read out the actual color and not the printed word as quickly as possible. The amount of time taken (seconds) to complete each set of words was recorded. This task assesses executive function.

2.4.4. *Urine sample collection and preparation*

Urine samples were collected at baseline prior QGPJ consumption and thereafter were collected in sterilized urine containers over the following time periods: 0-2, 2-6, 6-12, and 12-24 hours after QGPJ consumption. The volume of collected urine samples was measured per container and recorded, and an aliquot of 30 mL of urine sample plus 9 mL of formic acid (100%) was stored in 50-mL tubes, with additional 10 mL of urine for storage. The urine samples were stored at –80°C for batch analysis. Intact (nonmetabolized) QGP anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) as well as their main/common conjugated and methylated metabolites such as peonidin-glycosides and -glucuronides were determined by high-performance liquid chromatography photodiode array detection mass spectrometry method as described by Netzel et al (2012) [36].

2.5. *Statistical analyses*

Data were analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics of participant characteristics were performed across age groups. Normal distribution of the continuous variables was assessed using the Shapiro-Wilk test, histogram, Q-Q plot, and skewness and kurtosis.

Linear mixed modeling was used to estimate the effect of different timed doses of QGPJ on BP between the 2 age groups while adjusting for correlation due to repeated observations on each participant over 24 hours. Age-group and dose (with interaction term) were entered in the model as fixed effects while controlling for age and sex. Maximum likelihood method of estimation was used with a diagonal covariance structure. As BP measurements were collected over 24 hours, there were a few missing data (less than 1%), and as a result, linear mixed modeling was chosen for analysis because it handles missing data better than the widely used analysis of covariance.

Area under the curve (AUC) was calculated as a summation measure for the first 6 hours of BP measurements, and the baseline observation carried forward approach was used for missing data (less than 1%). To determine if the plum juice had a significant effect on BP, a z test was used to analyze the AUC for the BP. A series of t tests was used to determine whether there was any significant difference between baseline BP and different time points up to 6 hours. The period between 0 and 6 hours represents the time that the participants spent at the study facility under standard resting conditions.

One-way analysis of variance (ANOVA) was used to determine differences in performance within each group at baseline and 6 hours postintervention, and 2-way ANOVA was used to determine the difference in cognitive performance at baseline and 6 hours between the 2 age groups and dosing regimens as well as anthocyanin excretion between the 2 age groups.

3. Results

3.1. *Subject characteristics*

Twenty-four participants (12 young and 12 old adults) were recruited to participate in the study, and sociodemographic characteristics are presented in Table 1. Participants were of

Caucasian descent (n = 21), of African descent (n = 2), and Asian (n = 1). All participants attended both visits, and there were no withdrawals or adverse events reported throughout the study protocol. The average washout period for participants was 20 days, a deviation from the original 2-week washout period due to schedule clashes and illness. The washout period chosen for our study was 2 weeks. This was informed by the FDA recommendation which states that, for bioequivalence studies, "The washout time should be approximately 10× the plasma apparent terminal elimination half-life, to provide for 99.9% of the administered dose to be eliminated from the body." [45]. For the anthocyanin, cyanidin-3-glucoside, which is the main anthocyanin in QGPJ [36,46], the half-life has been shown to range between 12 and 51 hours [47].

3.2. *Twenty-four–hour ambulatory BP*

Hourly cardiovascular responses recorded during each of the 24-hour test periods are shown according to plum juice delivery mode in Figs. 1-4. Comparison is made between the dosing regimen (single and triple doses) and age groups with an interaction factor (dosing regimen × age group). Fig. 1a shows a more obvious drop in SBP of the older adults with the single dose compared to the triple dose (Fig. 3a). This observation was not evident with the younger adults, as shown in Figs. 1a and 3a. There was no significant dose-timing effect observed for change in BP following plum juice consumption in the 24-hour period using the linear mixed model for longitudinal data (Table 2).

AUC was calculated for the cardiovascular parameters (systolic, diastolic, mean arterial pressure [MAP], and heart rate [HR]) for the first 6 hours (Figs. 5-6 and Table 3). For both age groups, using a *t* test, BP was significantly lower than baseline ($P < .05$) at different time points up to 6 hours following consumption of the plum juice. The greatest significant BP reduction was observed at 2 hours for both age groups and was more obvious for SBP in the older group with a mean difference of 12.83 mm Hg (SD; 16.51, $P = .001$) from baseline. For the single dose, z test analysis of the AUC calculations for the younger adult group showed a significant effect of the juice on DBP, MAP, and HR (P values = .008, .012, and .025 respectively). Similarly, a significant effect was seen for the older group: SBP, DBP, MAP, and HR (P values = .035, .028, .017, and .006 respectively). For the younger age group on the triple dose, significant effects were observed for DBP and MAP (P values = .008 and .013, respectively) with a borderline significance on the HR (P value = .06). In the older group, significant effects of the triple dose were observed for DBP (P value = .00007) and a borderline effect for SBP (P value = .063). Plum juice consumption had a significant effect on SBP, which was predicted by dose or age group but no interaction term effect (dose × age group) and for MAP, predicted by only age group. No significant effect of plum juice was observed on other cardiovascular parameters (Table 2).

3.3. *Cognitive tasks*

Using 2-way ANOVA, a significant difference was observed between the 2 age groups ($P < .001$), both at baseline and 6 hours, for performance on cognitive tests. After consumption of the juice, there was no significant difference from baseline values within the groups or by dose timing.

3.4. *Urinary excretion of anthocyanins and anthocyanin metabolites*

The anthocyanin content of the batch of QGP used for our study was 123 mg/100 g. The consumption of QGPJ as a single oral dose of 300 mL or in 3 × 100-mL servings over 3 hours

Table 1 – Information about the subjects

Characteristics	Younger adults (n = 12) n (%)	Older adults (n = 12) n (%)	P values
Sex			
Male	4 (33.3)	3 (25.0)	NS
Female	8 (66.7)	9 (75.0)	
Age (means ± SD)	31 (8)	77 (6)	<.001
BMI (means ± SD)	22.5 (2.4)	26.4 (3.3)	.003
Physical activity			
Low	5 (41.7)	1 (8.3)	.97
Medium	5 (41.7)	10 (83.3)	
High	2 (16.7)	1 (8.3)	
Smoking status			
Yes	0 (0)	0 (0)	NS
No	11 (91.7)	12 (100)	
Occasionally	0 (0)	0 (0)	
Rarely	1 (8.3)	0 (0)	
Alcohol intake			
Yes	3 (25.0)	5 (41.7)	.67
No	2 (17.7)	3 (25.0)	
Occasionally	4 (33.3)	2 (16.7)	
Rarely	3 (25.0)	2 (16.7)	

Data are means ± SD or n (%) (n = 12). *P* values were obtained from χ^2 test for categorical variables.
Abbreviations: BMI, body mass index; NS, not significant.

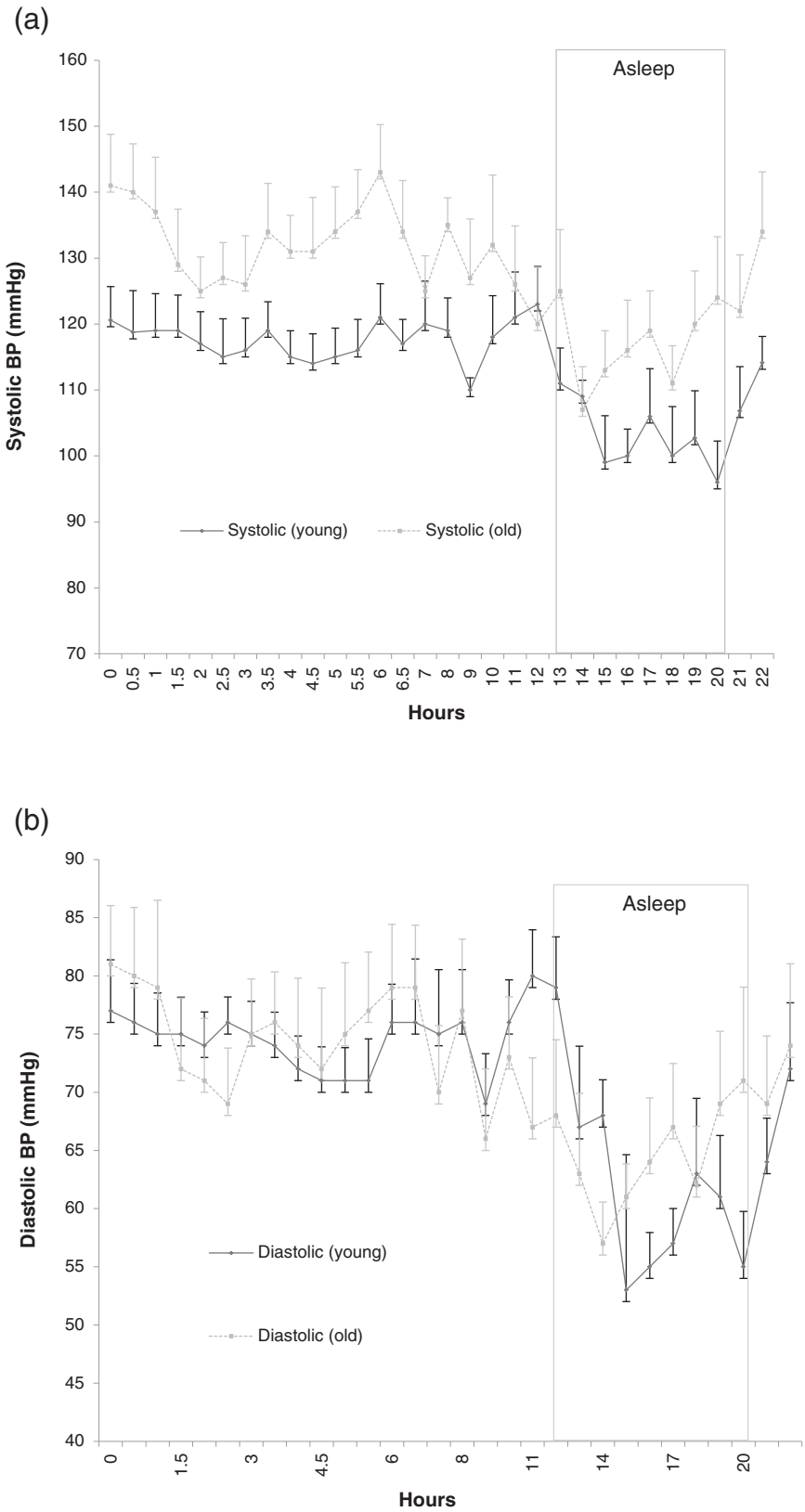

Fig. 1 – a, Single-dose hourly SBP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). BP indicates blood pressure; QGP, Queen Garnet plum. b, Single-dose hourly DBP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). BP indicates blood pressure; QGP, Queen Garnet plum.

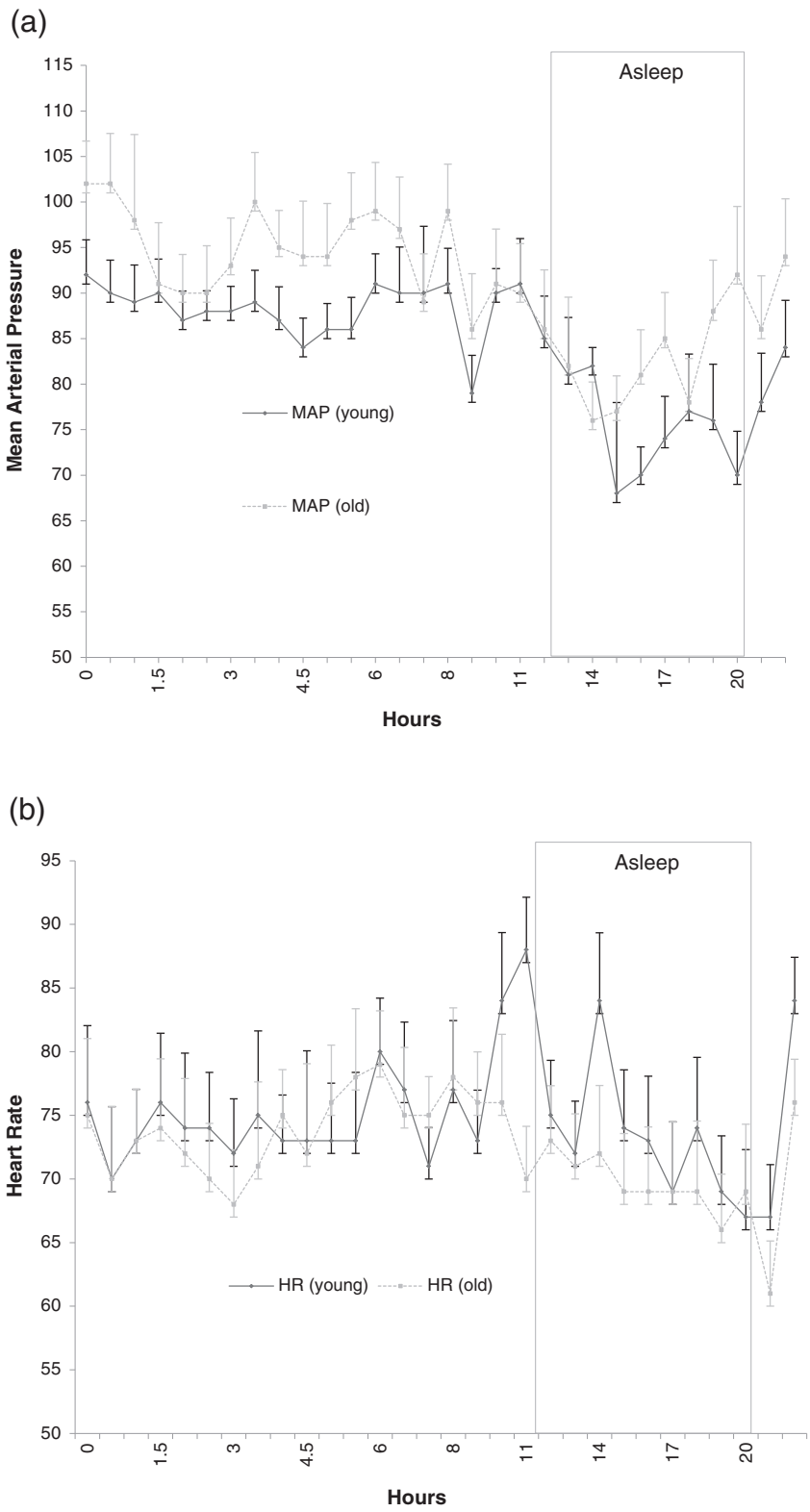

Fig. 2 – a, Single-dose hourly mean arterial BP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). MAP indicates mean arterial pressure; QGP, Queen Garnet plum. b, Single-dose hourly heart rate of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). HR indicates heart rate; QGP, Queen Garnet plum.

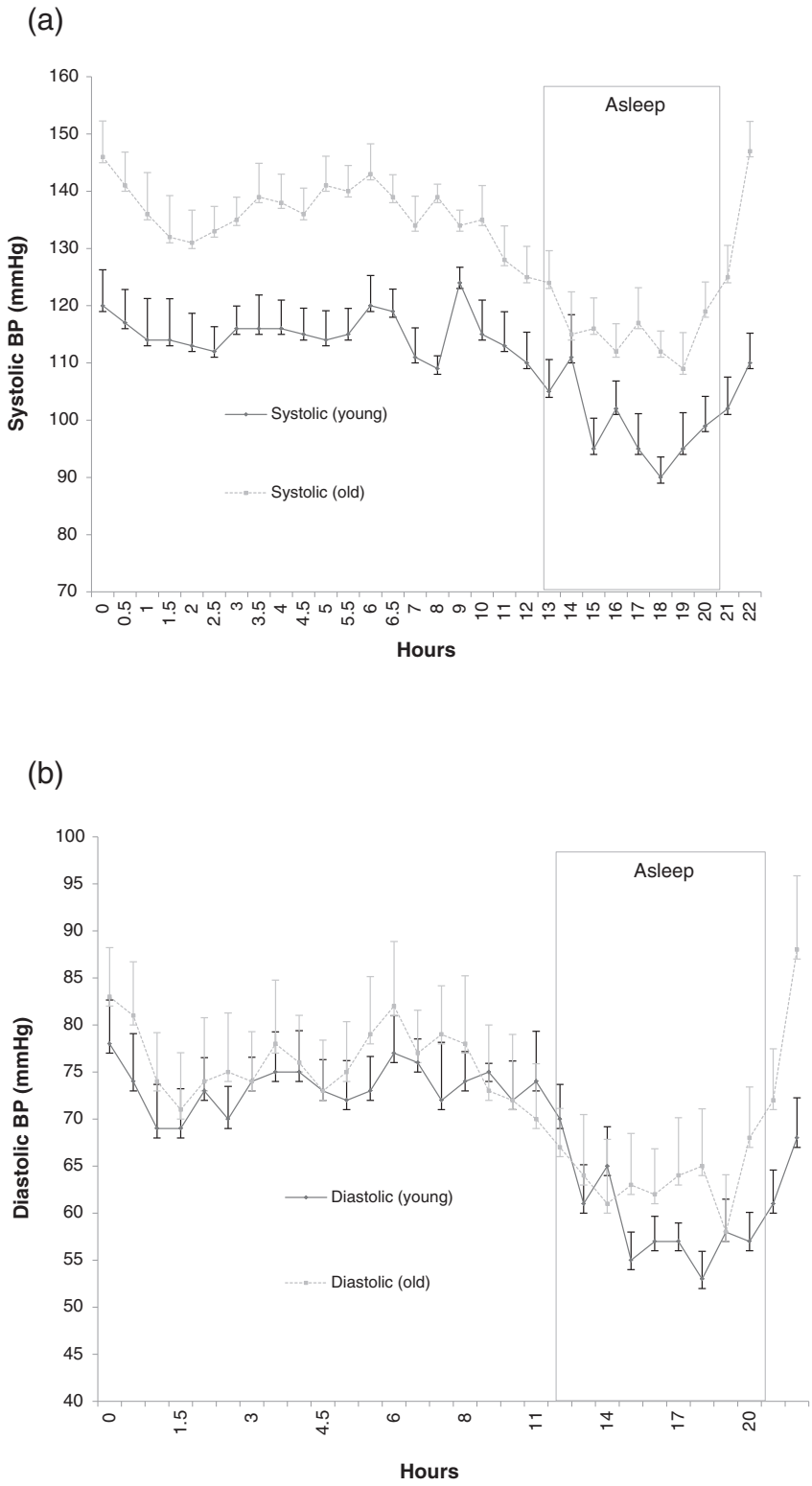

Fig. 3 – a, Triple-dose hourly SBP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). BP indicates blood pressure; QGP, Queen Garnet plum. b, Triple-dose hourly DBP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). BP indicates blood pressure; QGP, Queen Garnet plum.

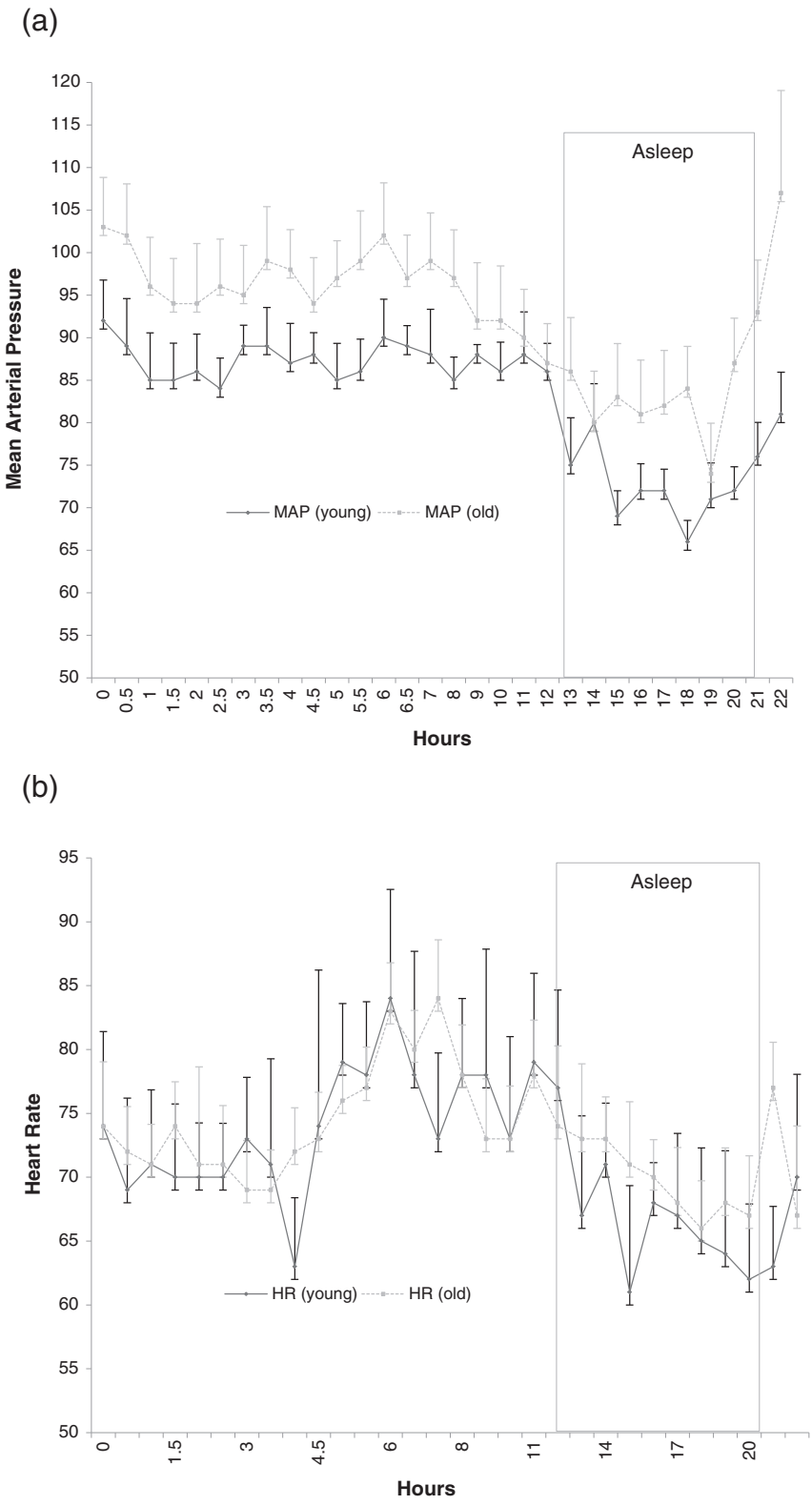

Fig. 4 – a, Triple-dose hourly mean arterial BP of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). MAP indicates mean arterial pressure; QGP, Queen Garnet plum. b, Triple-dose hourly HR of participants over 24 hours after consumption of QGPJ. Values are expressed as mean values ± SE (error bars) (n = 12 per age group). HR indicates heart rate; QGP, Queen Garnet plum.

Table 2 – Effect of dose-timed QGPJ consumption on cardiovascular parameters across age groups

Parameter	Mean ± SE	*P* values
SBP		
Intercept	123.7 ± 0.05	<.0001 [a]
Dose-time		
1 × 300 mL	122.3 ± 0.60	.001 [a]
3 × 100 mL	125.2 ± 0.67	
Group		
Younger	115.0 ± 0.66	<.0001 [a]
Older	132.8 ± 0.61	
Interaction (group * dose)		.154
Younger * 1 × 300 mL	114.2 ± 0.84	
Younger * 3 × 100 mL	115.8 ± 1.00	
Older * 1 × 300 mL	130.3 ± 0.87	
Older * 3 ×100 mL	134.5 ± 0.87	
DBP		
Intercept	72.9 ± 0.36	<.0001[a]
Dose-time		
1 × 300 mL	72.5 ± 0.49	
3 × 100 mL	73.3 ± 0.54	.25
Group		.11
Younger	72.2 ± 0.53	
Older	73.6 ± 0.49	
Interaction (group * dose)		
Younger * 1 × 300 mL	72.0 ± 0.68	.62
Younger * 3 × 100 mL	72.4 ± 0.81	
Older * 1 300 mL	73.0 ± 0.69	
Older * 3 × 100 mL	74.1 ± 0.70	
MAP		<.0001[a]
Intercept	90.2 ± 0.37	
Dose-time		.077
1 × 300 mL	89.4 ± 0.49	
3 × 100 mL	90.9 ± 0.54	
Group		<.0001[a]
Younger	86.4 ± 0.54	
Older	94.0 ± 0.50	
Interaction (group * dose)		.75
Younger * 1 × 300 mL	85.7 ± 0.68	
Younger * 3 × 100 mL	87.0 ± 0.83	
Older * 1 × 300 mL	93.1 ± 0.70	
Older * 3 × 100 mL	94.9 ± 0.70	
HR		
Intercept	72.6 ± 0.36	<.0001[a]
Dose-time		.69
1 × 300 mL	72.5 ± 0.49	
3 × 100 mL	72.7 ± 0.54	
Group		.76
Younger	72.9 ± 0.54	
Older	72.4 ± 0.49	
Interaction (group * dose)		.91
Younger * 1 × 300 mL	72.8 ± 0.69	
Younger * 3 × 100 mL	73.0 ± 0.82	
Older * 1 × 300 mL	72.2 ± 0.70	
Older * 3 × 100 mL	72.5 ± 0.70	

Values are means ± SE (n = 12 per group).
Means and *P* values were obtained from linear mixed model.
"Younger" means younger age group, and "older" means older age group.
Dose-time represents either a single dose of 300 mL or 3 portions of 100 mL taken at 0, 1, and 3 hours.
[a] Significant at $P < .05$.

resulted in the appearance of both intact/nonmetabolized QGP anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) and at least 5 identified anthocyanin metabolites in the volunteers' urine samples (Table 4). The excretion rates and urinary anthocyanin/metabolite profiles were similar ($P > .05$) between age groups and dosing regimen.

4. Discussion

Following consumption of a single dose of 300-mL dose of plum juice, an acute reduction in SBP ($P = .035$), DBP ($P = .028$), MAP ($P = .017$), and HR ($P = .006$) was observed in the older age group. A similar trend was also observed for the triple dose with the absence of an effect on SBP. This acute effect was more pronounced in the older age group and at 2 hours with a mean difference of 12.83 mm Hg from baseline. Significant effects on DBP ($P < .001$) and MAP ($P = .013$) were also observed in the younger age group on the single dose and DBP ($P < .001$) with a borderline effect on SBP ($P = .06$) on the triple dose. The acute significant reduction in BP at 2 hours is associated with evidence on the absorption and bioavailability of anthocyanins that occur within 2 hours postconsumption [48]. Anthocyanin concentrations in the body have been observed to reach peak levels between 1 and 2 hours and begin to clear from 6 hours, falling back to baseline levels as they get excreted from the body up to 48 hours [49]. The synergistic effect of other nutrients in the QGP cannot be overlooked. There is a possibility that the observed BP-lowering effect may have been as a result of this synergistic effect, as well as the presence of potassium in the QGP fruit [30] which is an electrolyte known to lower BP in humans [50]. Despite lack of a significant effect of dose by the different age groups on BP, the greater reduction in BP in response to plum juice consumption in older adults may be explained by their higher baseline BP levels [4]. However, further adequately powered studies are needed to confirm these findings. In the 6 hours following the plum juice consumption, no significant effect was observed on the SBP of the younger age group. Similar observation has also been made in previous studies. A study by Novotny et al [51] observed that the Pacific Kids Dietary Approach to Stop Hypertension trial did not affect overall diet quality which was measured by SBP change among other parameters but had a significant effect on DBP by the end of the intervention, by 12.2 mm Hg. There is a possibility that the absence of a significant effect on SBP could be associated with age because there is a possibility that interventions may have a more significant effect among older populations and/or those more prone to age-related vascular stiffening with an increased risk of developing CVD.

There was an observed dose timing and group effect on SBP but not on other BP parameters; however, this was no longer significant after inclusion of an interaction term (age group × dose timing). Previously, a similar study observed an acute reduction in BP (SBP, DBP, and HR) after consumption of anthocyanin-rich cherry juice, which was found to be dose-timing dependent [31]. The difference between the 2 studies may be explained by a much higher concentration of anthocyanins in the QGPJ (123 mg/100 mL vs 69 mg/100 mL, respectively), resulting in a physiological threshold to be reached in each of the three 100-mL doses.

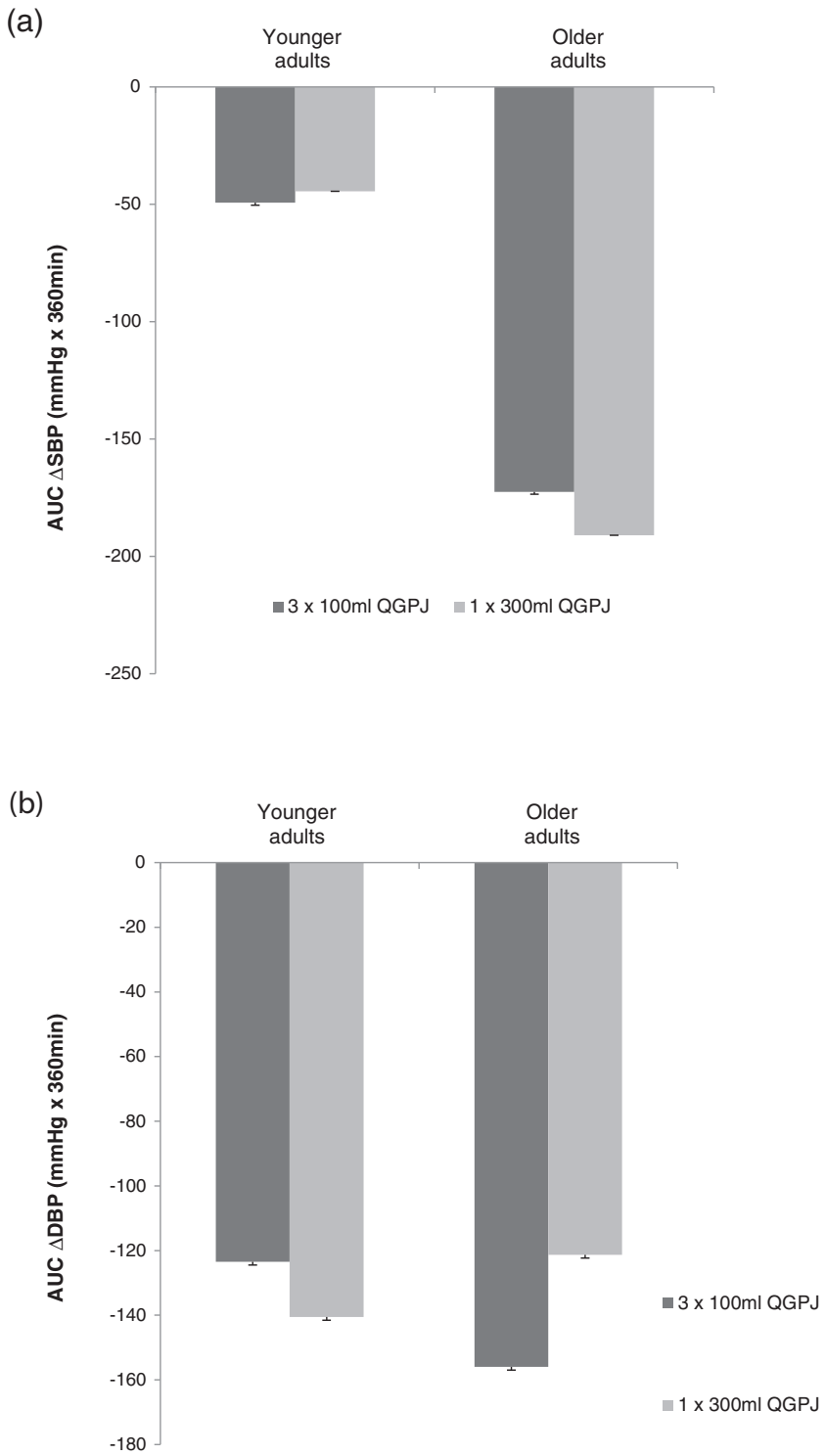

Fig. 5 – a, Change in SBP (0-6 hours) following consumption of QGPJ. Values are expressed as AUC for mean change in SBP from baseline per hour up to 6 hours. Bars represent the sum of AUC for ΔSBP (0-6 hours) ± SE (n = 12 per age group). AUC indicates area under the curve; QGPJ, Queen Garnet plum juice; SBP, systolic blood pressure. b, Change in DBP (0-6 hours) following consumption of QGPJ. Values are expressed as AUC for mean change in DBP from baseline per hour up to 6 hours. Bars represent the sum of AUC for ΔDBP (0-6 hours) ± SE (n = 12 per age group). AUC indicates area under the curve; QGPJ, Queen Garnet plum juice; DBP, diastolic blood pressure.

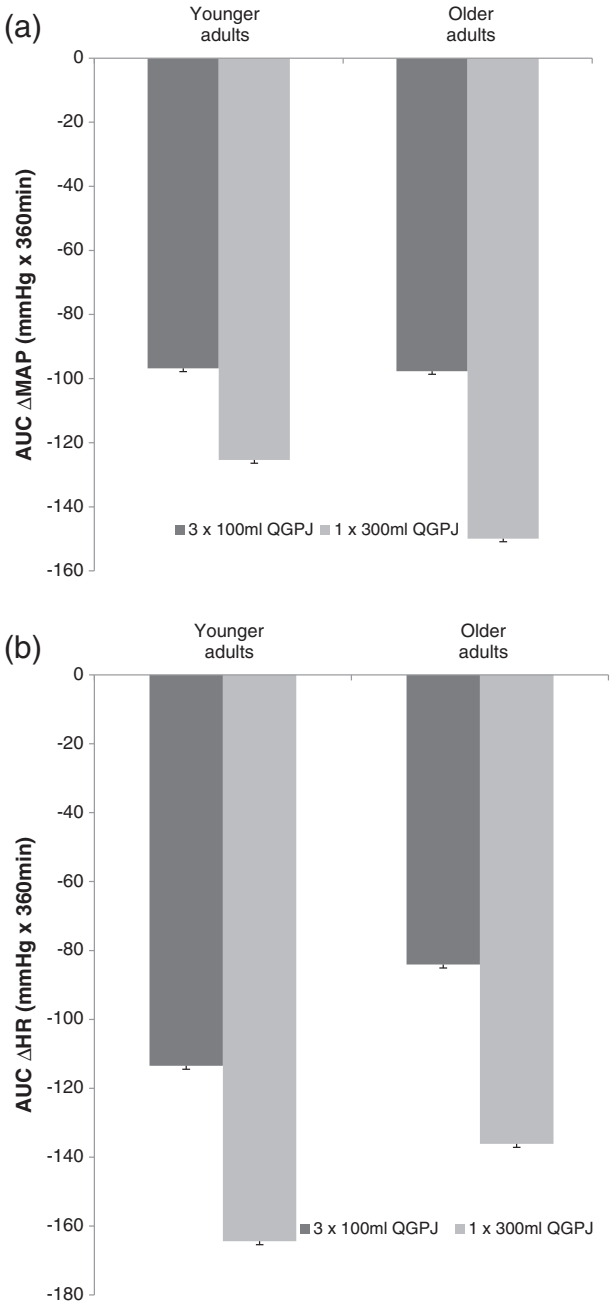

Fig. 6 – a, Change in MAP (0-6 hours) following consumption of QGPJ. Values are expressed as AUC for mean change in MAP from baseline per hour up to 6 hours. Bars represent the sum of AUC for ΔMAP (0-6 hours) ± SE (n = 12 per age group). AUC indicates area under the curve; QGPJ, Queen Garnet plum juice; MAP, mean arterial pressure. b, Change in heart rate (0-6 hours) following consumption of QGPJ. Values are expressed as AUC for mean change in HR from baseline per hour up to 6 hours. Bars represent the sum of AUC for ΔHR (0-6 hours) ± SE (n = 12 per age group). AUC indicates area under the curve; QGPJ, Queen Garnet plum juice; HR, heart rate.

A possible explanation/mechanism of the observed BP-lowering effect of QGPJ could lie in the molecular structure of its main in vivo anthocyanin metabolites. The described methylation of cyanidin glycosides by catechol-O-methyltransferase to peonidin-based metabolites as the main urinary anthocyanin metabolites after QGPJ ingestion results in a structural modification of the B-ring in the flavonoid skeleton which is structurally analogous to apocynin, an established vasoactive drug [8,52,53]. Mono-O-methylated anthocyanins/flavonoids can act as inhibitors of nicotinamide adenine dinucleotide phosphate oxidase and, as a result, can improve vasodilatory processes [8].

Another possible explanation for the BP-lowering effect of anthocyanins and/or other polyphenols present in the plum juice is their potential to inhibit the oxidation of low-density lipoproteins (LDLs), a major risk factor for atherosclerosis, through free-radical scavenging and removal of metal ions from catalytic sites via chelation [54]. The mechanism by which oxidized LDL promotes atherogenesis is believed to be through cytotoxicity, inhibitory effects on macrophage motility, and uptake by the macrophage scavenger receptor resulting in stimulation of cholesterol accumulation and hence foam cell formation, which is critical in early atherosclerosis lesions. To test this theory, Liu et al [55] in their study observed that when cells were incubated with oxidized LDL (100 μg/mL) for 24 hours, there was an increase in cell death, whereas the additions of mulberry water extracts and mulberry anthocyanin-rich extracts beyond the concentrations of 0.1 and 0.05 mg/mL, respectively, significantly increased the survival of these cell macrophages. They also observed that 1 mg/mL of mulberry water extracts and 0.1 mg/mL of mulberry anthocyanin-rich extract suppressed the lipid accumulation by approximately 55% and 58%, respectively.

Although anthocyanins have been hypothesized to promote healthy brain functioning, results from our pilot study show that a 300-mL serving of plum juice, regardless of dose timing or age of participants, has no significant acute effect on various domains of cognitive function. Although previous studies have found no significant acute effect of anthocyanins from fruit source on cognitive processes, the QGPJ used in the present study had a significantly higher content of anthocyanins, and therefore, there was a possibility that it might induce cognitive effects. In addition, 2 different cognitive tests that have been shown to be sensitive and target different domains were used: Stroop and the Reaction Time task [39,56]. Extensive research has been carried out on the long-term effect of flavonoid supplementation on cognition [39] with less attention on their acute effects. Recently, there has been an increase in the body of evidence on the acute effects of flavonoids on cognitive processes such as attention, working memory, and psychomotor speed in a general population [57]. The precise mechanism by which anthocyanins affect cognition is still not clear but seems to be dependent on the exposure period. Acute effects on cognition are believed to be as a result of increased cerebrovascular blood flow and possibly MAO inhibition which has been shown to improve cognitive performance [14,20]. Following consumption of high-anthocyanin fruit/juice, evidence shows that peaks in cerebral blood flow, vasodilation, and anthocyanin metabolite availability is detectable within 2 hours postconsumption [17]. Following blueberry supplementation, plasma anthocyanins and their metabolites were observed to reach peak levels at 1-2 and 6 hours [11]. An investigation on the bioavailability of anthocyanins observed an association

Table 3 – Change in cardiovascular parameters of participants following consumption of different doses of QGPJ

Cardiovascular parameters	AUC (0-6 h), mm Hg	
	3 × 100 mL QGP (0, 1, 3 h)	1 × 300 mL QGP
Younger adults (18-45)		
SBP	−49.36 ± 131.26	−44.45 ± 125.29
DBP	−123.45 ± 122.27 [a]	−140.55 ± 175.93 [a]
MAP	−96.80 ± 12.75 [a]	−125.40 ± 136.31 [a]
HR	−113.50 ± 208.69 [b]	−164.42 ± 253.64 [a]
Older adults (65+)		
SBP	−172.50 ± 321.86 [b]	−191.00 ± 313.93 [a]
DBP	−156.00 ± 136.28 [a]	−121.33 ± 190.81 [a]
MAP	−97.67 ± 278.55	−149.92 ± 217.91 [a]
HR	−84.08 ± 195.42	−136.17 ± 171.35 [a]

Values are AUC for mean change in cardiovascular parameters ± SD (n = 12 per group).
Abbreviations: AUC, area under the curve; BP, blood pressure; QGP, Queen Garnet plum.
[a] The z test analysis showed statistically significant effect of the juice on the measured parameter.
[b] The z test analysis showed borderline significant effect of the juice on the measured parameter.

between colon microbiota metabolism of anthocyanins and a significant increase in the content of generated polyphenols in the brain. There is a possibility that the peak levels observed at 6 hours are as a result of reuptake of polyphenols in the colon [58,59]. For this reason, repeat cognitive tasks were administered 6 hours postconsumption of the plum juice for our study. The absence of a significant effect could be attributed to the timing of cognitive task administration, missing the initial peak action time.

The urinary recovery of intact anthocyanins and anthocyanin metabolites that had an intact flavonoid skeleton (glucuronides, sulfates, and methylated forms) was between 693 and 871 μg/24 h, corresponding to 0.19%-0.24% of the ingested anthocyanin dose. These ranges are consistent with those reported in human studies for urinary excretion rates of anthocyanins and conjugated/methylated metabolites after consumption of anthocyanin-rich food (0.01%-5.10%) [7,36,60]. The bioavailability of anthocyanins has been reported to be low; however, a recent review indicates that it may be higher than previously reported [61]. Evidence from this review showed that most ingested anthocyanins reach the large intestine. Here, they are catabolized by the microbiota, producing an array of phenolic components that are absorbed, and some metabolized to phase II conjugates [61]. Furthermore, our finding that methylated and glucuronidated derivatives of cyanidin-based anthocyanins were the main urinary metabolites is also in agreement with others [62-64]. The in vivo glucuronidation, sulfatation, and methylation of anthocyanins by uridine diphosphate-glucuronosyltransferases, sulfotransferase, and catechol-O-methyltransferase in the intestinal epithelial cells, liver, and kidney are a common metabolic pathway of dietary anthocyanins and other polyphenolic compounds [65].

The presence of pelargonidin monoglucuronide, when QGPJ does not contain any (detectable) pelargonidin-based anthocyanins, can be explained by the in vivo xenobiotic and gut bacterial metabolism of anthocyanins/flavonoids. This includes addition and removal of methyl and hydroxyl groups (pelargonidin is lacking of one hydroxyl group compared with cyanidin) [66]. Furthermore, Kalt and colleagues [66] reported similar results. After the ingestion of blueberry juice, which did not contain any detectable pelargonidin glycosides, significant amounts of pelargonidin-based metabolites could be detected in the urine of 17 study subjects [66]. The described interconversion of anthocyanins due to xenobiotic and bacterial metabolism was suggested by these authors. There were no differences in the urinary anthocyanin/metabolite excretion profiles either between the age groups or according to the different dosing regimens (1 × 300-mL dose or 3 × 100-mL servings).

Previous research has reported conflicting results regarding the influence of flavonoids on cognition in younger and older people. In one study, anthocyanin-rich blueberry supplementation in younger and older adults resulted in

Table 4 – Urinary excretion of anthocyanins and anthocyanin metabolites in different age groups following the consumption of QGPJ as a single oral dose of 300 mL or as three 100-mL servings

	Dosing (anthocyanins)	Absolute excretion (μg/24 h) [a]	Relative excretion (%) [b]	Relative excretion of main metabolites (%) [c]
Younger age group	1 × 300 mL (369 mg)	811 ± 702	0.22	80
	3 × 100 mL (123 mg/dose)	759 ± 358	0.21	74
Older age group	1 × 300 mL (369 mg)	871 ± 602	0.24	80
	3 × 100 mL (123 mg/dose)	693 ± 458	0.19	75

Values are means ± SD (n = 12 per group).
[a] Sum of cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-glucoside monoglucuronide, cyanidin monoglucuronide, peonidin-3-glucoside, peonidin monoglucuronide, and pelargonidin monoglucuronide.
[b] Excreted amount vs ingested dose.
[c] Excreted amount of peonidin-3-glucoside and peonidin monoglucuronide as the main metabolites vs absolute excretion. Compounds were analyzed by high-performance liquid chromatography photodiode array detection mass spectrometry method and quantified by an external cyanidin-3-glucoside calibration curve [36].

improvements in different acute cognitive domains, whereby a significant improvement in updating ability was reported for younger adults and improvements in immediate word recognition in older adults were identified [67]. In relation to cocoa flavonoids, consumption of dark chocolate for 1 week significantly improved endothelial function and reduced BP in younger hypertensive patients but not in older populations [68]. Overall, there is little information that compares responses between younger and older adult populations; thus, more work comparing these groups is required to elucidate any age-related differences in biological response.

The main objective with the dose-timing design was to estimate the response according to the dose given to analyze the effect and identify any adverse reactions. Throughout the course of the study, the juice was well tolerated, and there were no reports of any adverse effects; however, the tolerability to the study protocol was not objectively measured. As there is large observed interindividual variation in the absorption, metabolism, and excretion of polyphenols [69], the use of a crossover study design is appropriate because participants act as their own controls [70].

4.1. *Limitations of the study*

A notable limitation of our study is the absence of a placebo arm. While a placebo arm is essential in dietary intervention studies to identify the magnitude of effect related to the dietary factor of interest, in the case of anthocyanins, Johnson et al [71] included a placebo control group in their blueberry powder (469 mg of anthocyanins per day) study and identified a drop of 7 mm Hg and 5 mm Hg after 8 weeks of intervention in both SBP and DBP ($P < .05$ and $P < .01$, respectively) but not in the control group. The main purpose of our acute study in which each participant acted as their own control was to identify whether different dosing regimens of a high-anthocyanin fruit juice resulted in differences in cognitive performance and/or BP. Information related to the dose-timing administration of an intervention is an important consideration in clinical trial designs in free-living participants. Furthermore, neither intact anthocyanins nor their common metabolites such as glucuronides, sulfates, or methylated forms are usually detectable in urine of placebo/control groups as demonstrated in a pilot study with QGPJ and water as a control [36]. Food or beverages used for placebo/control treatments are usually anthocyanin free or contain only negligible amounts of these pigments.

Previous long-term flavonoid trials have instructed participants to consume an amount of food or beverage over the period of a day but without specific guidelines on whether this needs to be consumed in totality at a single setting or whether smaller portions can be spread across the day. Nonspecific information on timing of the test food or beverage probably relates to a poor understanding of how dose timing may affect biological responses.

Another notable limitation is the absence of a double-blinded strategy. This, in addition to cognitive testing time, could have resulted in the absence of a significant effect on cognitive performance after consumption of the plum juice. A consideration for future studies could be to test cognitive effects 2 hours and 4-6 hours postconsumption to reflect metabolic processes and thus consolidate available evidence. Another important consideration for future clinical trials may be to screen for individuals with arterial narrowing who may benefit most from blood vessel dilation related to dietary interventions [72,73]. There is a possibility that a greater BP response would result in more pronounced cognitive functioning, which was not evident in the current study. In addition, it is recommended that blood and fecal samples are included in future human studies to allow a more comprehensive analysis of in vivo metabolites specifically generated by the gut microbiota and thereby elucidate the mode of action of these plant bioactives.

In conclusion, our research hypothesis was not accepted because there were no differences according to 2 dose-timing regimens of consumption of QGPJ. An acute BP-lowering effect of anthocyanin-rich plum juice was similarly observed for both dose-timing regimens, whereas no cognitive effects were observed for either dose, nor were differences in anthocyanin metabolite excretion evident between younger and older adults. Anthocyanin metabolites were bioavailable in urine following consumption, but no differences were observed in the absorption rate and metabolism of anthocyanins between young and older adults assessed in urine. It is important that the mechanism of action is studied further to better understand how anthocyanins exert protective effects on BP and how this reduction effect can be sustained over time, as well as the effects on cognition in longer-term consumption studies. With more significant effects observed in older participants, future studies should focus on this age group where elevated BP is more prevalent by using a placebo-controlled design.

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