Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults[1–3]

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ABSTRACT

Background: Research indicates that the chronic consumption of flavonoids is associated with cognitive benefits in adults with mild cognitive impairment and neurodegenerative disease, although to our knowledge, there have been no such studies in healthy older adults. Furthermore, the effects of commonly consumed orange juice flavanones on cognitive function remain unexplored.

Objective: We investigated whether 8 wk of daily flavanone-rich orange juice consumption was beneficial for cognitive function in healthy older adults.

Design: High-flavanone (305 mg) 100% orange juice and an equicaloric low-flavanone (37 mg) orange-flavored cordial (500 mL) were consumed daily for 8 wk by 37 healthy older adults (mean age: 67 y) according to a crossover, double-blind, randomized design separated by a 4-wk washout. Cognitive function, mood, and blood pressure were assessed at baseline and follow-up by using standardized validated tests.

Results: Global cognitive function was significantly better after 8-wk consumption of flavanone-rich juice than after 8-wk consumption of the low-flavanone control. No significant effects on mood or blood pressure were observed.

Conclusions: Chronic daily consumption of flavanone-rich 100% orange juice over 8 wk is beneficial for cognitive function in healthy older adults. The potential for flavanone-rich foods and drinks to attenuate cognitive decline in aging and the mechanisms that underlie these effects should be investigated. This trial was registered at clinicaltrials.gov as NCT01312610. *Am J Clin Nutr* 2015; 101:506–14.

Keywords cognitive function, flavanone, flavonoid, orange juice, executive function

INTRODUCTION

There is significant interest in the impact of dietary interventions for maintaining cognitive function in old age and delaying the onset of neurodegenerative diseases. Over the past decade, numerous studies showed an association between a group of phytochemicals known as flavonoids and benefits for cognitive function in humans (1, 2). Epidemiologic research indicated that higher intakes of flavonoid-rich foods and drinks over 10–15 y are associated with a reduced rate of cognitive decline in old age (3, 4). Acute interventions that ranged from a single dose to daily doses over 12 wk showed that short-term cognitive benefits can be achieved from increased flavonoid consumption. For example, acute doses of cocoa flavanols were associated with improvements in executive function (5) and spatial memory (6) 2 h postconsumption in healthy young adults. Berries and grapes are a rich source of the flavonoid subclass known as anthocyanins. Several studies reported that 12 wk of daily consumption of grape juice (7, 8) or blueberry juice (9) is associated with benefits for memory in older adults with mild cognitive impairment.

The mechanisms by which flavonoids and their subclasses may affect cognitive function remain to be clearly elucidated; however, the growing body of positive findings in humans is encouraging and supported by a wealth of animal data indicating that flavonoid-rich foods improve age-related deficits in memory (10–13). The aforementioned studies primarily examined flavanols and anthocyanins, and there has been very little investigation into the flavonoid subclass known as flavanones. This is a notable absence because one of the most-commonly consumed juices throughout the world (orange juice) is a rich source of flavanones. Moreover, flavanones are one of the most-easily absorbed flavonoids and known to cross the blood-brain barrier (14). A cross-sectional epidemiologic survey reported a positive association between flavanone consumption and crystallized intelligence (15), and rodents pretreated with the flavanone hesperidin were less likely to exhibit impairments in locomotor activity and hippocampal function after the onset of Huntington's disease, indicating that flavanones may offer neuroprotective effects (16). Although evidence for acute, beneficial cognitive effects of flavonoid-rich foods and drinks exists in older populations with mild cognitive impairment, to our knowledge, there have been no such interventions in healthy

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older adults. Therefore, the aim of the current study was to conduct a well-controlled, placebo-matched, crossover, randomized, double-blind, human-intervention trial that examined the effect of 8 wk of daily consumption of flavanone-rich orange juice on cognitive function in healthy older adults.

METHODS

Thirty-seven fluent English speakers (24 women; 13 men) aged 60–81 y (mean ± SD age: 66.7 ± 5.3 y) were recruited from the University of Reading Older Adults Research Panel and Hugh Sinclair Adults Volunteer Panel and via community advertising with posters leaflets and adverts on British Broadcasting Corporation Berkshire Radio (**Table 1**). Participants were excluded for diagnosed type 2 diabetes, diagnosed hypertension (or blood pressure measurement >150/90 mm Hg at screening), dementia (Mini-Mental State Examination <26) (17), gastrointestinal abnormalities, cardiovascular disease, previous head injury, diagnosed mental illness including depression [Beck Depression Inventory (18) administered at screening], or prescribed long-term medication (however; a prescription of statins was not considered an exclusion criterion; $n = 5$). Previous crossover research showed significant cognitive effects of flavonoid consumption in older adults with mild cognitive impairment with sample sizes ranging from 9 to 21; however, because our older adult sample would not have mild cognitive impairment, we targeted a sample size of 40 to avoid being underpowered. One participant withdrew for personal reasons during the first arm (low-flavonoid drink). Recruitment commenced in October 2010 and terminated in December 2011. This trial was registered at clinicaltrials.gov as NCT01312610.

Design

A double-blind, randomized, crossover design was applied with 2 drink conditions as follows: high flavanone (HF)[4] and low flavanone (LF). The HF drink contained 549 mg hesperidin/L and 60 mg narirutin/L (producing a daily 500-mL serving of 305 mg flavanones), and the LF drink contained 64 mg hesperidin/L and 10 mg narirutin/L (producing a daily 500-mL serving of 37 mg flavanones). These flavanone concentrations were natural to the product, and no flavanones were artificially added. The HF drink contained 180 kcal/500-mL daily serving (44.9 g total sugars), and the LF drink contained 165 kcal/500-mL daily serving (41 g total sugars). Participants were instructed to drink 250 mL twice per day for 8 wk. This volume of juice was chosen to achieve a balance between a habitual diet and meaningful flavanone dose. Previous research has shown significant effects on cognitive function in healthy older adults after doses ranging from 60 to 768 mg/d (2), and 2 portions of 250 mL/d represents an achievable volume of consumption within the context of the habitual diet. The HF drink contained one part 100% orange juice to 3 parts water. The orange juice was produced and canned by Florida Citrus with 100% oranges and stored frozen. Participants were required to add the 3 parts water to the HF orange juice. The LF drink contained one part Rocks Organic Orange Squash (Rocks Drinks) to 5 parts water. The LF drink was bottled and produced by Rocks Drinks. Participants were required to add 5 parts water to the LF drink. Participants were provided with written and oral instructions by a research assistant (RA) to prepare both drinks. Eighteen participants consumed LF and then HF drinks, and 19 participants consumed HF and then LF drinks (defined as the drink order). The order of random assignment was determined with Microsoft Excel (Microsoft Corp.) by the RA who did not administer test-day procedures. RJK and GFD enrolled participants and completed test-day procedures (notwithstanding the drink allocation), whereas the RA assigned participants to drink conditions, received and stored the drinks, and administered drinks to participants. There were slight differences in the packaging of each product; therefore, at the point of freezing, all labels for both drinks were removed and replaced with a code (by the RA), and thus, participants and experimenters remained blinded. RJK and GFD had no contact with the drinks and, therefore, remained blinded until completion of the analysis at which point the RA unblinded all experimenters.

TABLE 1
Demographic characteristics collected at screening[1]

	Men (n = 13)	Women (n = 24)
Age, y	67.6 ± 1.6	66.2 ± 1
MMSE (maximum of 30)	28.6 ± 0.4	29.1 ± 0.3
NART (maximum of 50)	39.5 ± 3.1	38.4 ± 1.4
Education, y	13.8 ± 1.1	12.8 ± 0.5
Systolic blood pressure, mm Hg*	142.4 ± 4.7	131.6 ± 2.8
Diastolic blood pressure, mm Hg*	82.9 ± 1.6	75.7 ± 1.5
BMI, kg/m^2	26.1 ± 1.1	26.3 ± 0.9
Weight, kg	78.2 ± 3.2	71.2 ± 2.3

[1]All values are means ± SEs. *Significantly greater for men than women, $P < 0.05$ (independent-samples t test). MMSE, Mini-Mental State Examination; NART, National Adult Reading Test.

Procedure

Initially, telephone screening interviews were performed, and volunteers who met the inclusion criteria were invited to attend the Hugh Sinclair Nutrition Unit for a screening visit. At screening, data on height, weight, health status, medication, and blood pressure were collected, and participants completed the Mini-Mental State Examination and National Adult Reading Test. Before each visit, participants were instructed to fast from alcohol for 48 h and avoid polyphenol-rich foods for 24 h (including berries; fruits; fruit juices; jams and preserves; red wine; black, green, and fruit teas; coffee; cocoa; soy products; caffeinated energy drinks; and vegetables except potatoes) and were provided with standardized typed instructions that identified which foods to avoid. For all visits, participants arrived fasted at 0800. Participants were required to orally confirm that they had adhered to the aforementioned dietary restrictions. After a 15-min rest, blood pressure measurements were taken on the left upper arm by using a validated blood pressure monitor (Omron MX2 Digital Automatic Upper Arm Blood Pressure Monitor; OMRON) and recorded as the average of 3 consecutive

[4] Abbreviations used: BDNF, brain-derived neurotrophic factor; CBF, cerebral blood flow; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSST, Digit Symbol Substitution Test; HF, high flavanone; LF, low flavanone; RA, research assistant; VPA, Verbal Paired Associates.

measurements. At 0830, participants consumed a standardized breakfast [100 g croissant, 20 g cream cheese, and 25 g rice-based cereal (Kellogg's), and 220 g semi-skimmed milk]. Subjective mood was assessed by using the Positive and Negative Affect Scale (19) immediately before commencement of the cognitive battery at 0900am. After 8 wk of juice consumption, participants returned for visit 2, which followed identical procedures to visit one. Participants commenced a 4-wk washout during which instructions were to consume a normal diet, notwithstanding the fasting restrictions before visit 3. Habitual diet was not assessed during the washout. After the 4-wk washout, participants returned for visit 3 which signified the beginning of the second drink condition. In sum, participants were tested at weeks 0 (visit 1), 8 (visit 2), 12 (visit 3), and 20 (visit 4). At the end of visits 1 and 3, participants were provided with an 8-wk supply of either the LF or the HF drink in accordance with counterbalancing. At the beginning of visits 2 and 4, a drink record was returned on which participants recorded the time of daily drink consumption. At the end of the final visit, participants were asked to indicate which drink they believed was the HF drink. Seventeen participants responded "I don't know," 16 participants correctly indentified the HF drink, and 4 participants incorrectly chose the LF drink. Therefore, less than one-half of participants were able to correctly identify the HF drink. The procedures were in accordance with the ethical standards of the University of Reading.

Cognitive tests

The following tests were administered by blinded researchers in the respective order; Go-NoGo (20), Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (immediate verbal recall) (21), Letter Memory (22), Verbal Paired Associates (VPA) (23) (immediate), CERAD (delayed verbal recall) (21), Serial Sevens (24), Spatial Working Memory (SWM) (25), Digit Symbol Substitution Test (DSST) (26), Letter Fluency, DSST and Letter Fluency (both tests performed simultaneously), and VPA (delayed). Matched, equal versions of each test were assigned to visits in a counterbalanced order. These tests were grouped into 2 cognitive constructs of executive function or episodic memory as described by (27, 28).

Executive function tests

The Go-NoGo is a computerized task that assesses inhibition and sustained attention. The current version was adapted from the Go-NoGo paradigm (20) and previously showed sensitivity to flavonoid consumption (29). Participants were required to respond to 60 stimuli by using 1of 3 specified keyboard keys as follows; p, q, or space bar. The stimuli consisted of X, Y, or a number lure. Initially, there was a 25-stimuli prepotent Go phase. During the prepotent Go phase, X and Y were presented alternately with the participant required to press q when X appeared and p when Y appeared. The X and Y were known as the Go trials. The Go-NoGo phase followed the prepotent Go phase. During the Go-NoGo phase, Go trials were interspersed with NoGo trials; these appeared as numbers lures. Pressing the space bar was the required response on viewing a number lure. During the Go-NoGo phase, X and Y were presented randomly and interspersed with number lures such that the predictable alternating sequence was disrupted. Responses were required only if a Y appeared after an X or vice versa, and therefore, the participant must have inhibited the established prepotent response in all other trials. The reaction time for correct responses was the dependent variable. Serial Sevens required participants to continuously subtract 7 from a randomly generated number between 700 and 1000 for 2 min. In the case of incorrect responses, subsequent responses were scored as correct in relation to the new number. The dependent variable was the number of correct responses. The DSST (26) is a pen and paper test that contains a key of 9 digit-symbol pairs and an accompanying list of digits. Under each listed digit, a space is provided for the participant to enter the corresponding symbol. The task was to enter as many symbols as possible over 90 s. The dependent variable was the number of correct responses. For the Letter Fluency task, participants had 1 min to name as many words as possible beginning with an orally presented letter avoiding proper nouns, pluralization, function words, and repetition. Three trials were administered with each trial being a different letter. The dependent variable was the average number of words produced per trial. The Letter Memory Task (22) involved a serial 2000-ms presentation of individual letters. The number of letters per trial varied randomly between 5, 7, 9, and 11 for a total of 12 trials and 48 letters. For each trial, at the termination of the presentation phase, participants were required to orally recall the final 4 letters from the presentation. The dependent variable was the total number of correct responses defined as recalling the correct sequence in its entirety. The Letter Memory test predominately requires the updating component (30) of executive function of the central executive and is, therefore, categorized as a test of executive function rather than episodic memory (22).

Episodic memory tests

The CERAD (21) involved 3 consecutive oral presentations of 15 words by the experimenter at a rate of one word every 2 s. Immediately after the presentation of each list, participants orally recalled the words in any order. The dependent variable was the average number of words recalled per trial. For delayed recall, participants were asked to recall the previously presented words. There were 2 stages to the VPA (23) test of encoding and recognition. At encoding, 16 semantically unrelated word pairs were serially presented at a rate of one pair per second, and participants were instructed to covertly generate a sentence including all of the words. At recognition, 32 words pairs were presented, 16 of which were presented at encoding (targets) and 16 of which contained one original word paired with a new word (distracters). The task was to identify the targets, and the dependent variable was the number of targets correctly recognized. For delayed VPA, the recognition stage was repeated. For the Spatial Working Memory Test (25), a 250-ms blank screen was followed by the appearance of a black square for 1250 ms in 1 of 20 possible locations (randomly ordered). This sequence of appearing and disappearing black squares continued for 72 trials. A response was required (target) when the square appeared in a location in which it had previously appeared. Each version contained 12 targets, and the dependent variable was the number of correctly identified targets.

Statistical analysis

To consider the effect of the drink order on all cognitive performance and subjective mood-dependent variables, a 3-factor

TABLE 2
Cognitive test and subjective mood measure values[1]

Dependent variable	Low-flavanone drink	High-flavanone drink	*P* across drinks
Tests of executive function			
DSST (n = 36)			
Baseline	56.6 ± 2.4	56.8 ± 2.4	0.94
8 wk	58.4 ± 2.1	59.9 ± 2.1	0.23
Change from baseline	1.8 ± 1.6	3.1 ± 1.5	0.58
DSST dual (n = 36)			
Baseline	29.4 ± 1.5	28.6 ± 1.6	0.63
8 wk	30.6 ± 1.4	29.4 ± 1.5	0.33
Change from baseline	1.1 ± 1.2	0.8 ± 1.3	0.87
Go-NoGo RT (n = 34), ms			
Baseline	550 ± 13	550 ± 11	0.71
8 wk	554 ± 11	546 ± 11	0.2
Change from baseline	4 ± 10	−4 ± 9	0.54
Letter Fluency (n = 36)			
Baseline	29.6 ± 1.2	28.8 ± 1	0.35
8 wk	28.2 ± 1.1	29.6 ± 1.2	0.14
Change from baseline	−1.4 ± 0.9	0.8 ± 1	0.08
Letter Fluency dual (n = 36)			
Baseline	12.9 ± 0.7	12 ± 0.6	0.28
8 wk	11.5 ± 0.6	12.2 ± 0.5	0.34
Change from baseline	−1.4 ± 0.6	0.2 ± 0.7	0.16
Letter Memory (n = 35) (maximum of 12)			
Baseline	4.3 ± 0.3	4.4 ± 0.4	0.63
8 wk	4.5 ± 0.4	4.9 ± 0.5	0.28
Change from baseline	0.2 ± 0.3	0.5 ± 0.4	0.5
Serial Sevens (n = 36)			
Baseline	14.7 ± 1.4	14.3 ± 1.3	0.84
8 wk	15.1 ± 1.4	16.4 ± 1.6	0.17
Change from baseline	0.5 ± 1.2	2.1 ± 1.1	0.22
Tests of episodic memory			
CERAD immediate (n = 35) (maximum of 15)			
Baseline	8.8 ± 0.4	9.1 ± 0.3	0.17
8 wk	9.1 ± 0.4	9.6 ± 0.4	0.03*
Change from baseline	0.26 ± 0.28	0.5 ± 0.25	0.54
CERAD delayed (n = 35)			
Baseline	7.9 ± 0.6	7.9 ± 0.7	0.9
8 wk	7.6 ± 0.7	8.1 ± 0.7	0.29
Change from baseline	−0.3 ± 0.4	0.2 ± 0.6	0.39
Spatial Working Memory (n = 34) (maximum of 12)			
Baseline	8.2 ± 0.4	7.9 ± 0.4	0.4
8 wk	7 ± 0.4	7.5 ± 0.4	0.14
Change from baseline	−1.2 ± 0.4	−0.4 ± 0.3	0.06
Verbal Paired Associates immediate (n = 31)			
Baseline (maximum of 16)	12.4 ± 0.4	13 ± 0.3	0.57
8 wk	12.5 ± 0.4	12.7 ± 0.4	0.99
Change from baseline	0.1 ± 0.5	−0.3 ± 0.4	0.6
Verbal Paired Associates delayed (n = 28)			
Baseline (maximum of 16)	11.2 ± 0.4	11.8 ± 0.4	0.32
8 wk	10.5 ± 0.5	11.4 ± 0.4	0.54
Change from baseline	−0.8 ± 0.5	−0.5 ± 0.5	0.65
Subjective mood measures			
Positive affect (PANAS) (n = 33) (maximum of 5)			
Baseline	3.5 ± 0.1	3.4 ± 0.1	0.31
8 wk	3.5 ± 0.1	3.4 ± 0.1	0.36
Change from baseline	0 ± 0.1	0 ± 0.1	0.89
Negative affect (PANAS) (n = 33) (maximum of 5)			
Baseline	1.49 ± 0.1	1.52 ± 0.1	0.84
8 wk	1.18 ± 0.1	1.25 ± 0.1	0.3
Change from baseline	−0.31 ± 0.1	−0.27 ± 0.1	0.71

[1]All values are means ± SEs. Data for cognitive tests are correct responses unless stated otherwise. *$P < 0.05$. CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSST, Digit Symbol Substitution Test; PANAS, Positive and Negative Affect Scale; RT, reaction time.

mixed ANOVA was performed with the drink order (LF and then HF; HF and then LF), drink (LF and HF), and visit (baseline and 8 wk) as independent variables in the model. Subsequently, post hoc tests (repeated-measures *t* tests) with Bonferroni corrections for family-wise error were performed by examining significant main effects and interactions where appropriate (corrected *P* values are reported). In addition, for consistency, interpretability, and agreement with other studies (28), cognitive tests were grouped into 2 cognitive constructs of executive function and episodic memory. Initially, the grand mean across both drinks and time points for each cognitive test was calculated and converted into *z* scores. For each time point (baseline and 8 wk) and drink (LF and HF), a mean *z* score was calculated such that

$$\text{Executive function} = (z_{\text{DSST}} + z_{\text{Go-NoGo}} + z_{\text{Serial Sevens}} + z_{\text{Letter Fluency}} + z_{\text{Letter Memory}} + z_{\text{dual DSST}} + z_{\text{dual Letter Fluency}}) \div 7 \quad (1)$$

and

$$\text{Episodic memory} = (z_{\text{immediateCERAD}} + z_{\text{delayedCERAD}} + z_{\text{immediateVPAL}} + z_{\text{delayedVPA}} + z_{\text{SWM}}) \div 5 \quad (2).$$

Global cognitive function was calculated with same method by combining all cognitive tests. For the Go-NoGo reaction time, data *z* scores were multiplied by −1 because a higher score represented worse performance. There were 2 dependent variables for the Positive and Negative Affect Scale subjective mood measure as follows: positive affect and negative affect (19). Blood pressure data were analyzed by using a 3-factor mixed ANOVA with sex (male and female), drink (LF and HF), and visit (baseline and 8 wk) as independent variables in the model. The analysis was performed with SPSS Statistics 19 software (SPSS Inc.).

RESULTS

Cognitive function

Raw data for each dependent variable are shown in **Table 2**, and results from the 3-factor ANOVA are shown in **Table 3**. The ANOVA model revealed a significant drink × visit interaction for global cognitive function (all tests combined) ($F_{[1,35]} = 4.53$, $P < 0.05$). As shown in **Figure 1**, post hoc tests (with corrections for 2 comparisons) revealed that global performance was significantly better after the HF drink than after the LF drink at 8 wk ($t = 2.86$, df = 38, $P < 0.05$), whereas no significant difference was observed between drinks at baseline. Similarly, a significant drink × visit interaction was observed for executive function ($F_{[1,35]} = 4.2$, $P < 0.05$). As shown in Figure 1, this interaction was explained by improvement at 8 wk after the HF drink; however, the post hoc test was NS ($P = 0.06$). The ANOVA model revealed a significant main effect of drink for episodic memory ($F_{[1,38]} = 5.55$, $P < 0.05$) such that, when collated across time points, memory was better with the HF drink (mean ± SE *z* score: 0.074 ± 0.1) than LF drink (mean ± SE *z* score: −0.054 ± 0.09); however, the drink × visit interaction was NS ($P > 0.05$). These results indicated that episodic memory was better for the HF drink condition at baseline (before drink consumption) and at 8-wk follow-up. As shown in Table 3, the drink × visit interaction was NS for any of the individual tests

TABLE 3
Significance values from the 2 × 2 × 2 ANOVA model (drink order × drink × visit) for each dependent variable[1]

Dependent variable	Main effect of drink	Main effect of visit	Drink × visit interaction	Main effect of drink order
Cognitive construct				
Global cognitive function[2] (*n* = 37)	0.074	0.86	0.04*	0.33
Executive function[3] (*n* = 37)	0.4	0.14	0.048*	0.32
Episodic memory[4] (*n* = 37)	0.024*	0.18	0.37	0.5
Tests of executive function				
DSST (*n* = 36)	0.38	0.026*	0.65	0.44
DSST dual (*n* = 36)	0.38	0.19	0.74	0.71
Go-NoGo RT (*n* = 34)	0.35	0.98	0.52	0.013*
Letter Fluency (*n* = 36)	0.62	0.69	0.076	0.21
Letter Fluency dual (*n* = 36)	0.86	0.2	0.15	0.97
Letter Memory (*n* = 35)	0.27	0.22	0.52	0.76
Serial Sevens (*n* = 36)	0.45	0.14	0.31	0.89
Tests of episodic memory				
CERAD immediate (*n* = 35)	0.006**	0.048*	0.56	0.56
CERAD delayed (*n* = 35)	0.23	0.27	0.61	0.59
Spatial Working Memory (*n* = 34)	0.55	0.007**	0.069	0.74
VPA immediate (*n* = 31)	0.59	0.76	0.63	0.026*
VPA delayed (*n* = 28)	0.066	0.11	0.71	0.32
Subjective mood measures				
Positive affect (*n* = 33)	0.21	0.93	0.89	0.71
Negative affect (*n* = 33)	0.43	<0.001**	0.56	0.64

[1]CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSST, Digit Symbol Substitution Test; RT, reaction time; VPA, Verbal Paired Associates.*$P < 0.05$ and ** $P < 0.01$.
[2]*z* scores combined from all cognitive tests (not including subjective mood measures).
[3]*z* scores combined from tests of executive function.
[4]*z* scores combined from tests of episodic memory.

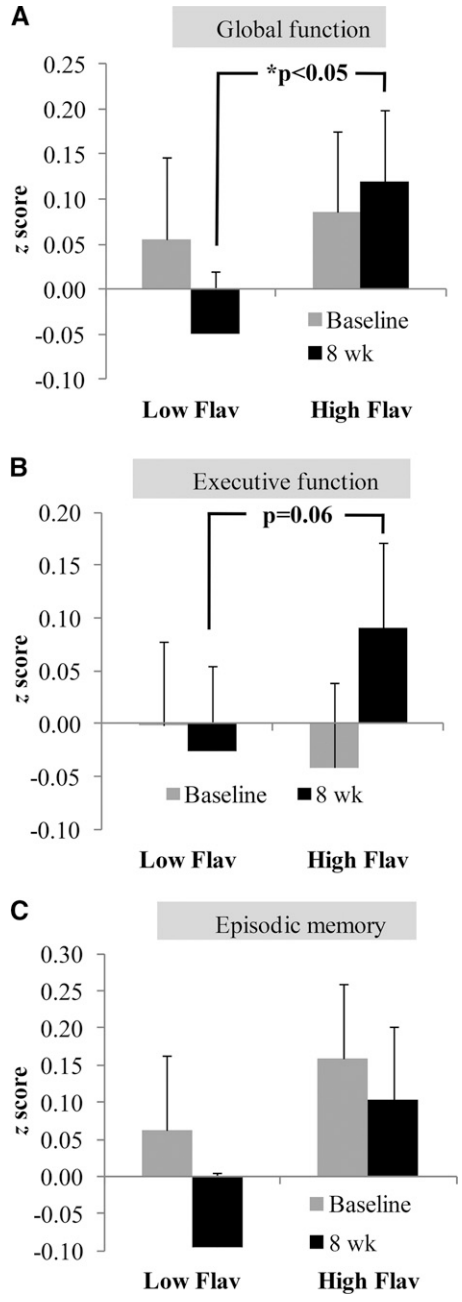

FIGURE 1 Mean z scores for global cognitive function, executive function, and episodic memory at baseline and 8 wk for Low Flav and High Flav drinks (n = 37). A significant drink × visit interaction ($P < 0.05$) in shown in panel A such that global function was significantly better after the High Flav than after the Low Flav drink at 8 wk (following post hoc t tests with Bonferroni corrections, $P < 0.05$). A significant drink × visit interaction ($P < 0.05$) for executive function is shown in panel B; however, post hoc tests were NS ($P = 0.06$). Finally, a main effect of drink for episodic memory is shown in panel C such that memory function was significantly greater for the High Flav drink than for the Low Flav drink across both time points; however, the drink × visit interaction was NS. For executive function outcome z score, data for correct responses were combined from the following cognitive tests: DSST, dual DSST, Go-NoGo (reaction time), Letter Fluency, dual Letter Fluency, Letter Memory, and Serial Sevens. For the episodic memory outcome z score, data for correct responses were combined from the following cognitive tests: CERAD immediate and delayed recall, Spatial Working Memory, and Verbal Paired Associates immediate and delayed recognition. Global cognitive function consisted of all these cognitive tests. CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSST, Digit Symbol Substitution Test; High Flav, high flavanone; Low Flav, low flavanone.

within the full ANOVA model; however, a main effect of drink was observed for CERAD immediate recall ($F_{[1,34]} = 8.65$, $P < 0.01$) such that better recall was observed with the HF drink (mean ± SE: 9.6 ± 0.4) than with the LF drink (mean ± SE: 9.1 ± 0.4). Similar to the composite episodic memory score, this result indicated that CERAD immediate recall was better for the HF drink condition at baseline (before drink consumption) and at 8-wk follow-up. The main effects of visit (Table 3) indicate that, regardless of which drink was consumed, performance was better at the 8-wk visit for the DSST and CERAD immediate recall but, conversely, better at the baseline visit for Spatial Working Memory (Table 3), suggesting that repeated testing was not consistently associated with improved performance. Overall, there were no main effects of visit across the vast majority of outcome measures, which indicated that practice effects accounted for a very small portion of variance in the model.

Effects of drink order

As shown in Table 3, the drink order did not significantly affect the vast majority of outcomes. However, 2 main effects (Go-NoGo and VPA immediate) revealed that performance was significantly better (across all visits and drinks) when the HF drink was consumed during the first arm than when the LF drink was consumed during the first arm (Go-NoGo reaction time means: HF first arm, 526 ms; LF first arm, 577 ms; VPA means: HF first arm, 13.2; LF first arm, 11.9). This result indicated potential carryover effects such that benefits associated with the HF drink may have persisted into the second arm.

Subjective mood, blood pressure, and weight

No significant effects were observed for positive affect. As shown in Table 2, a significant decline in negative affect was observed at 8 wk relative to baseline for the LF drink ($t = 3.76$, df = 32, $P < 0.01$) and HF drink ($t = 4.51$, df = 32, $P < 0.01$). This result was also reflected within the full ANOVA model as a highly significant main effect of visit (see Table 3). This finding indicated that negative affect significantly decreased across the duration of the trial regardless of which drink was consumed (means ± SEs: baseline visit, 1.5 ± 0.04; 8-wk visit, 1.21 ± 0.04). As shown in **Table 4**, there was no significant drink × visit interaction for systolic blood pressure or diastolic blood pressure. As expected, main effects of sex were observed for both systolic blood pressure ($F_{[1,37]} = 4.84$, $P < 0.05$) and diastolic blood pressure ($F_{[1,37]} = 6.27$, $P < 0.05$) such that women had lower blood pressure than men did (Table 4). In addition, a main effect of drink was observed for diastolic blood pressure ($F_{[1,37]} = 5.32$, $P < 0.05$) such that blood pressure was lower with the HF drink (mean ± SE: 76 ± 1.3 mm Hg) than with the LF drink (mean ± SE: 78 ± 1.4 mm Hg) when collated across time points and sexes, indicating that diastolic blood pressure was lower with the HF drink at baseline (before drink consumption) and at 8-wk follow up. Weight gain was observed over 8 wk with the LF drink (mean weight gain: 337 g) and the HF drink (496 g). Weight gain was not significantly different between the 2 drink conditions ($t = 0.57$, df = 26, $P = 0.57$).

DISCUSSION

The current findings showed that 8 wk daily consumption of flavanone-rich orange juice was associated with benefits for

TABLE 4
Blood pressure readings split by sex, drink, and visit[1]

	Men (n = 14)		Women (n = 27)	
	Low flavanone	High flavanone	Low flavanone	High flavanone
Systolic blood pressure,[2] mm Hg				
Baseline	132 ± 4.3	135 ± 5.6	126 ± 2.7	123 ± 3.2
8 wk	136 ± 4	133 ± 3.4	125 ± 4	124 ± 2.4
Diastolic blood pressure,[3] mm Hg				
Baseline	81 ± 2.6	79 ± 2.4	75 ± 1.6	73 ± 1.7
8 wk	82 ± 2.6	79 ± 2.4	76 ± 1.6	73 ± 1.6

[1]All values are means ± SEs.
[2]Drink × visit × sex interaction, P = 0.24; drink × visit interaction, P = 0.76; visit × sex interaction, P = 0.78; drink × sex interaction, P = 0.63; visit main effect, P = 0.57; drink main effect, P = 0.55; sex main effect, $P < 0.05$.
[3]Drink × visit × sex interaction, P = 0.83; drink × visit interaction, P = 0.57; visit × sex interaction, P = 0.7; drink × sex interaction, P = 0.77; visit main effect, P = 0.7; drink main effect, $P < 0.05$; sex main effect, $P < 0.05$.

cognitive function in healthy older adults relative to the consumption of an LF orange juice control. Global cognitive function was better after 8 wk of consumption of flavanone-rich juice relative to 8 wk of consumption of the LF control. Rather than improve cognitive function, the flavanone-rich juice attenuated a general decline in performance observed after 8 wk of consumption of the LF juice. These cognitive benefits were consistent with effects observed in rodents supplemented with a flavonoid-rich diet (10–13) and research in older adults with mild cognitive impairment. For example, 12 wk of daily grape juice consumption was associated with benefits for verbal but not spatial memory (7). Executive function was not examined in the previous grape and blueberry juice 12-wk interventions, and therefore, to our knowledge, this is the first study to show improvements in this cognitive construct after a flavonoid juice–based intervention. In support of these findings, acute doses of cocoa flavanols were associated with benefits for executive function 2 h postconsumption (5). The reduction in negative affect observed between the baseline and 8-wk visits suggested that participants were feeling more comfortable and at ease in the experimental environment as the trial progressed. This effect did not vary as a function of the drink consumed, which indicated that this subjective mood effect was unrelated to flavanones and unlikely to have accounted for the cognitive effects.

The current data showed that the cognitive benefits achieved from regular daily flavonoid consumption were not exclusive to adults with mild cognitive impairment or neurodegenerative disease. Moreover, to our knowledge, cognitive benefits after daily consumption of flavanones were not previously reported in humans. Positive effects for executive function are of particular relevance because performance on tasks that require these functions are known to deteriorate as a consequence of normal aging (31) as a result of structural changes that preferentially affect the hippocampus and prefrontal cortex (32). The current study does not provide data to support possible mechanisms that underlie positive cognitive effects. However, several potential mechanisms have been previously described (33, 34). Of the flavonoid subclasses, flavanones are one of the best absorbed and most effective at crossing the blood-brain barrier (14), and naringenin has been shown to localize in the brain after oral ingestion (35). Rodent research indicated that flavanones may have specific neuroprotective effects such as reducing nitric oxide levels and increasing the scavenging of free radicals (36). For example, pretreatment with hesperidin significantly attenuated oxidative damage and restored antioxidant enzyme activities in the frontal cortex, striatum, and hippocampus (16). These neuroprotective effects were observed to be beneficial only when in a state of cognitive impairment after the onset of Huntington's disease; treatment with flavanones did not provide any behavioral or neurochemical benefits for rodents in a healthy cognitive state. These processes could account for the attenuation of cognitive decline observed in the current study, particularly if flavanones are most likely to target the frontal cortex and hippocampus, which are areas of the brain that are essential for executive function and memory, respectively. In addition to their neuroprotective effects, flavanones may also enhance cognition by improving synaptic plasticity via increased brain-derived neurotrophic factor (BDNF). Spatial learning improvements in rodents after flavonoid-rich diets are known to be associated with increased hippocampal BDNF expression (12, 37). Morphologic changes stimulated by BDNF such as increases in neuronal spine density and neurogenesis (38) can lead to enhanced synaptic connections (39), which could potentially account for improvements in cognitive processes such as executive function after prolonged intake of flavanones over several weeks. The expression of BDNF decreases with age in humans (40), therefore, mechanisms that reverse this effect could attenuate cognitive decline. However, at present, the evidence for these mechanisms relies on rodent models, and as such, the expression of these pathways in humans remains speculative. The timescale of effect for these mechanisms also remains unknown; e.g., the duration over which positive cognitive effects may persist after the cessation of treatment or flavonoid ingestion is of interest and has implications for experimental design. The current data indicated possible carryover effects into the second arm when the HF juice was consumed during the first arm (Go-NoGo and VPA); however, for the vast majority of outcomes, an effect of drink order was not evident. Nevertheless, future research should carefully consider carryover effects, which may also shed light on underlying mechanisms.

In addition to the aforementioned direct central mechanisms, it is possible that flavanones may have cognitive benefits via secondary mechanisms such as improved cerebral blood flow (CBF). Human studies showed significant increases in peripheral and CBF several hours after cocoa-flavanol consumption (41, 42), and increased steady state–evoked potentials in posterior parietal

and central-frontal regions were observed after 30 d daily consumption of cocoa-flavanol drinks (43). To our knowledge, there are no published data examining peripheral or CBF in humans after flavanone consumption, and therefore, this mechanism requires investigation. These vascular effects are important because increased CBF has been associated with the proliferation of hippocampal neuronal cells (44); however, clear associations between flavonoid-induced increases in CBF and improvements in behavioral outcomes on tests of cognitive function have proved elusive. For example, Francis et al. (42) reported an increase in CBF during an attention-switching task after consumption of 172 mg cocoa flavanols relative to 13 mg cocoa flavanols but no effects on attention-switching performance were observed. An absence of effects on peripheral blood pressure does not necessarily undermine a CBF-based mechanism accounting for cognitive effects. It can be hypothesized that increased CBF occurs in response to increased neuronal activation induced by the onset of a cognitively demanding task, and as such, this is an acute mechanism that is potentially mediated by chronic enhancement and increased efficiency of the endothelium and related cells in the blood-brain barrier associated with vasodilatory processes. It does not necessarily follow that chronic changes in peripheral vascular function (such as improved blood pressure) will be observed under such circumstances.

In conclusion, clearly much work is required to examine the potential mechanisms that may underlie the association between flavonoid consumption and cognitive function. However, to our knowledge, the current study is the first human intervention trial to show cognitive benefits after flavanone consumption. The consumption of flavanone-rich orange juice over 8 wk was associated with benefits for global cognitive function in healthy older adults. Future research should investigate the potential for flavanone-based dietary interventions to maintain and enhance cognitive function in healthy young adults and attenuate cognitive decline in older adults with neurodegenerative disease. Because of the increasing prevalence of ageing-associated neurodegenerative disease in Western populations, it is essential to identify dietary interventions that can be easily understood and adopted by the public.

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