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Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia

Katherine Caldwell
University of Wollongong, kc582@uowmail.edu.au

Karen E. Charlton
University of Wollongong, karenc@uow.edu.au

Steven J. Roodenrys
University of Wollongong, steven@uow.edu.au

Marijka Batterham
University of Wollongong, marijka@uow.edu.au

Jan M. Potter
Wollongong Hospital, janp@uow.edu.au

See next page for additional authors

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Abstract

Purpose Dietary flavonoids, including anthocyanins, may positively influence cognition and may be beneficial for the prevention and treatment of dementia. We aimed to assess whether daily consumption of anthocyanin-rich cherry juice changed cognitive function in older adults with dementia. Blood pressure and anti-inflammatory effects were examined as secondary outcomes. **Methods** A 12-week randomised controlled trial assessed cognitive outcomes in older adults (+70 year) with mild-to-moderate dementia (n = 49) after consumption of 200 ml/day of either a cherry juice or a control juice with negligible anthocyanin content. Blood pressure and inflammatory markers (CRP and IL-6) were measured at 6 and 12 weeks. ANCOVA controlling for baseline and RMANOVA assessed change in cognition and blood pressure. **Results** Improvements in verbal fluency ($p = 0.014$), short-term memory ($p = 0.014$) and long-term memory ($p \leq 0.001$) were found in the cherry juice group. A significant reduction in systolic ($p = 0.038$) blood pressure and a trend for diastolic ($p = 0.160$) blood pressure reduction was evident in the intervention group. Markers of inflammation (CRP and IL-6) were not altered. **Conclusion** Inclusion of an anthocyanin-rich beverage may be a practical and feasible way to improve total anthocyanin consumption in older adults with mild-to-moderate dementia, with potential to improve specific cognitive outcomes.

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Authors

Katherine Caldwell, Karen E. Charlton, Steven J. Roodenrys, Marijka Batterham, Jan M. Potter, Victoria Traynor, Hayley Gilbert, Olivia Morgan, and Rachelle Richards

Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild to moderate dementia

Katherine Kent[1], Karen Charlton[1], Steven Roodenrys[2], Marijka Batterham[3], Jan Potter[4], Victoria Traynor[5], Hayley Gilbert[2], Olivia Morgan[2], Rachelle Richards[2].

[1.] School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, NSW, Australia.

[2.] School of Psychology, Faculty of Social Sciences, University of Wollongong, NSW, Australia.

[3.] Statistical Consulting Service, University of Wollongong, NSW, Australia

[4.] Division of Aged Care and Rehabilitation, Illawarra Shoalhaven Local Health District, Wollongong Hospital, NSW, Australia

[5.] School of Nursing, Faculty of Science, Medicine and Health, University of Wollongong, NSW, Australia.

Abstract

Purpose: Dietary flavonoids, including anthocyanins, may positively influence cognition and may be beneficial for the prevention and treatment of dementia. We aimed to assess whether daily consumption of anthocyanin-rich cherry juice changed cognitive function in older adults with dementia. Blood pressure and anti-inflammatory effects were examined as secondary outcomes.

Methods: A 12-week randomised controlled trial assessed cognitive outcomes in older adults (+70y) with mild to moderate dementia (n=49) after consumption of 200ml/day of either a cherry juice or a control juice with negligible anthocyanin content. Blood pressure and inflammatory markers (CRP, IL-6) were measured at 6 and 12 weeks. ANCOVA controlling for baseline and RMANOVA assessed change in cognition and blood pressure.

Results: Improvements in verbal fluency (P=0.014), short term memory (P=0.014) and long term memory (P=<0.001) were found in the cherry juice group. A significant reduction in systolic (P=0.038) blood pressure and a trend for diastolic (P=0.160) blood pressure reduction was evident in the intervention group. Markers of inflammation (CRP and IL-6) were not altered.

Conclusion: Inclusion of an anthocyanin-rich beverage may be a practical and feasible way to improve total anthocyanin consumption in older adults with mild to moderate dementia, with potential to improve specific cognitive outcomes.

Introduction

Plant-based foods form an integral component of the human diet and their consumption is consistently linked to the maintenance of health and the prevention of a vast array of diseases (1). A growing body of evidence has shown that phytochemicals, non-nutritive bioactive compounds, contribute to the antioxidant activity of individual fruits and vegetables and are consequently credited with the observed health benefits (2). Flavonoids are a class of polyphenols that have been studied intensively and are categorised into six major classes: anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones (3). Flavonoids are found in particularly high concentrations in fruits and vegetables, wine, tea and cocoa (4). The consumption of flavonoids has been associated with a reduction in risk for cardiovascular diseases and some cancers (5, 6) and more recently there has been attention directed to their potential to protect against neurodegenerative diseases and improve cognitive performance in older adults (7).

The sub-groups of flavanols, anthocyanins and flavanones have been shown to be the most beneficial of the flavonoid family in terms of neuro-protection (8). Specifically, literature investigating the impact of fruit flavonoids on cognitive and physical functioning is predominately pre-clinical (9) and while promising, remains inconclusive. Much of the food-based research has focused on flavonoid-rich blueberries and strawberries (9). The biological actions of flavonoids on cognitive function have been attributed to a number of hypothesized mechanisms. Their antioxidant actions assist to scavenge free radicals in the brain (8), while their neuro-protective effects include protection of vulnerable neurons against inflammation, enhancement of existing neuronal function, increased blood flow to the brain and neurogenesis initiation in areas of the brain that are associated with cognition (10).

Preliminary dietary supplementation studies have shown that blueberries (11) and concord grapes (12, 13) improve aspects of memory in older adults over a 12 and 16 week intervention. Cherries, both the sweet and tart varieties, are a rich source of anthocyanins and to a lesser extent also contain flavan-3-ols and flavonols (14). Cherries have been found to lower inflammation and scavenge nitrous oxide radicals within the body (15). However the potential of cherry flavonoids to influence cognitive function has not been investigated, despite them being a commonly consumed fruit in Australia (4) and other countries.

Research to date has investigated the cognitive enhancing effects of flavonoid-rich foods in participants with both normal cognitive function (16) and people with mild cognitive impairment (11-13) but effects in dementia patients remains under researched (17). In light of projections indicating rapid increases in the prevalence of the dementia (18) and in the absence of successful treatments, alternative measures to slow the development and progression of dementia are imperative.

A randomised clinical trial was conducted to assess changes in cognitive function in older adults with mild to moderate dementia after daily consumption of a feasible serving of anthocyanin-rich cherry juice over 12 weeks. Secondary outcomes included anti-inflammatory effects, changes in functional and physical ability and mood.

Methods

Participants

Participants were recruited from a geriatric outpatient dementia clinic and residential aged care facilities in the Illawarra region of New South Wales, Australia. Participants were aged 70 years or older and had been diagnosed as having mild to moderate dementia Alzheimer's type, as confirmed by the consultant geriatrician responsible for their clinical management (JP). Exclusion criteria include non-English speaking, uncontrolled hypertension, uncontrolled diabetes, any other unstable physical or mental health condition or dysphagia. Written informed consent was obtained. For participants with cognitive impairment deemed by their geriatrician to be too severe to provide informed consent, consent was provided by family carers or guardians. Consenting participants (Table 1) were randomised to a treatment group and randomised by computer-aided block randomisation, conducted by a statistician that was independent to the research interface (MB) (Figure 1). All other researchers were blinded to the grouping. This study was approved by the University of Wollongong Human Research Ethics Committee and the Illawarra Shoalhaven Local Health Districts ethics committee (HE11/175). This study was registered as a clinical trial through the Australian New Zealand Clinical Trials Registry (ANZCTR) and was allocated the ACTRN: ACTRN12614001298606.

Study Procedure

Over 12 weeks participants received 200ml/day of cherry juice (intervention arm) or 200ml/day of commercially prepared apple juice (control arm). Juice was delivered weekly, by a research assistant not involved in data collection, to the homes of participants, chilled in

1L plastic bottles. Instructions were provided to participants and carers for the juice to be consumed daily, at any time in one sitting, using a cup provided that indicated 200ml with a fill line. A weekly calendar was provided with the juice, with daily check boxes ticked when the juice was consumed. The sweet, Bing cherry juice was produced using a novel method that aims to retain the phenolic bioactives, developed by a research company (Agritechnology) based in Orange, NSW, Australia. The high antioxidant activity and anthocyanin content of cherries is well retained in the juice which had a high Oxygen Radical Absorbance Capacity (3200 μmol Trolox Equivalents/g) and contained 69 mg red pigments (anthocyanins) per 100g of juice as determined by HPLC (19) (Table 2). The apple juice was provided by Appledale, based in Orange, NSW, Australia (Table 2). Nutritional content of the intervention and control juices are shown in Table 2. A serving size of 200ml per day was determined as being a feasible quantity that could be consumed by an older adult with mild-moderate dementia, and was chosen in the absence of empirical research that highlights a dose requirement for anthocyanin intake and cognitive outcomes. The serving size provided a more feasible serving of juice than has been used in previous similar studies, where the quantity of anthocyanin-rich beverages provided may exceed the amount that can be feasibly achieved over the long term, especially by older adults living with a neurodegenerative disease (11, 13). No change to regular diet was advised.

Table 1: Baseline characteristics of participants in the study according to group

Characteristic	Control group n=25, mean ± SD	Intervention group n=24, mean ± SD
Age (years)	80.6 ± 6.6	78.9 ± 5.2
BMI (kg/m^2)	26.6 ± 3.5	25.7 ± 3.4
Current smokers (count)	1 (4%)	0 (0%)
Previous smokers (count)	10 (40%)	7 (29%)
Hand grip strength (kg)	52.1 ± 16.4	62.5 ± 16.1*
Mid arm circumference (cm)	26.9 ± 4.5	27.2 ± 4.1
Calf circumference	33.4 ± 3.7	34.9 ± 3.4
Education (years)	17.5 ± 3.2	18.2 ± 2.4
Mini Nutritional Assessment	23.4 ± 3.5	25.6 ± 2.8*
Instrumental Activities of Daily Living	5.7 ± 2.4	7.5 ± 0.8*
Total flavonoid intake (mg/day)	429.8 ± 384.2	599.0 ± 352.2
Flavonol intake (mg/day)	20.0 ± 13.2	32.9 ± 26.5
Flavone intake (mg/day)	0.3 ± 0.3	1.2 ± 1.7
Flavanone intake (mg/day)*	8.8 ± 16.4	11.7 ± 17.9*
Flavon-3-ol intake (mg/day)*	382.9 ± 379.6	518.9 ± 338.9*
Anthocyanin intake (mg/day)	13.1 ± 29.7	34.2 ± 55.6*

*P<0.05, for differences between groups.

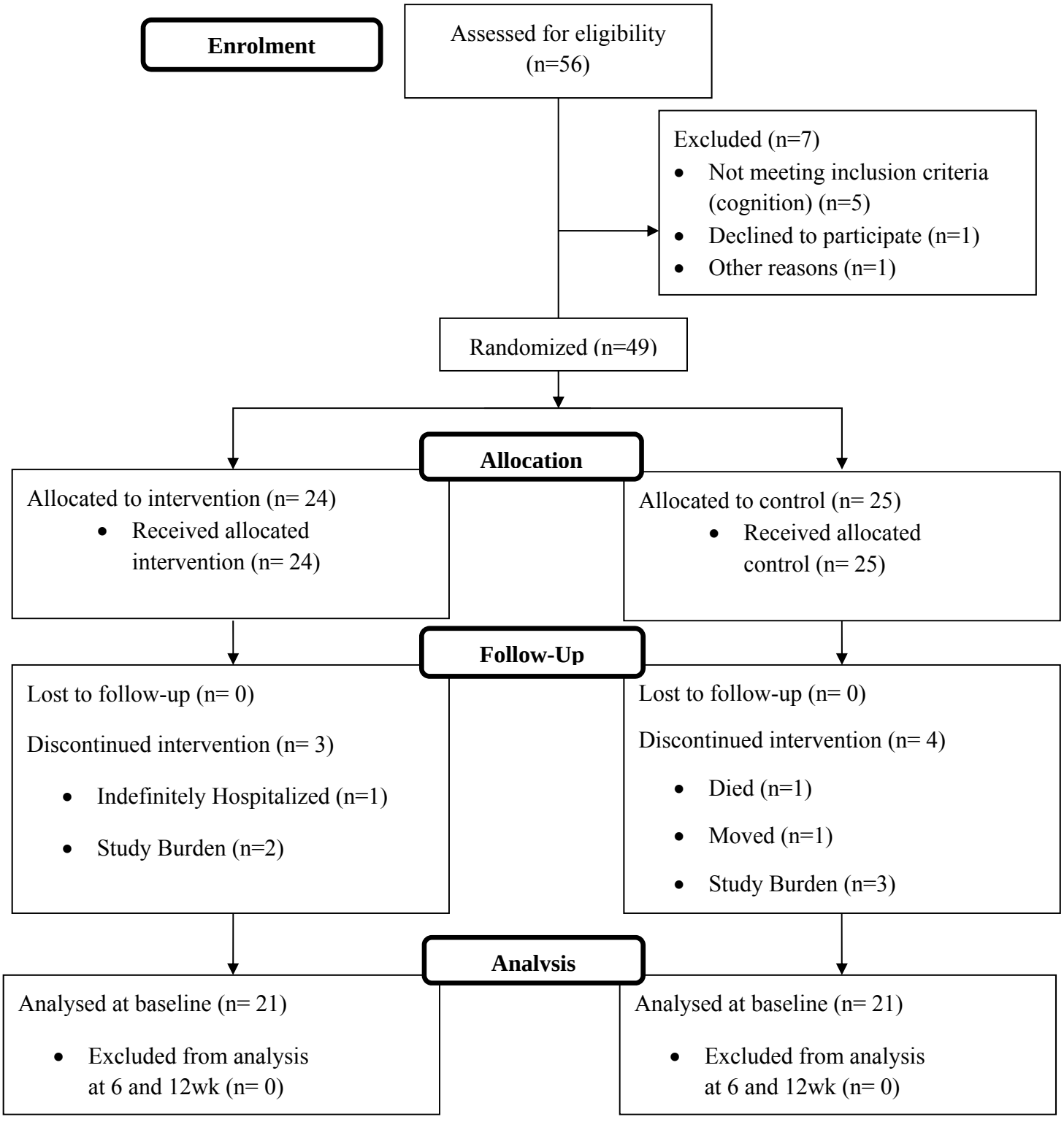

Figure 1. CONSORT flow diagram of enrolment, allocation, follow up and analysis.

Table 2: Nutritional properties of intervention and control juice

	Cherry Juice (100ml)	**Apple Juice** (100ml)
Energy	395kJ	180kJ
Protein	1.5g	0.1g
Fat Total	<0.2g	0.1g
Saturated	<0.2g	0.1g
Carbohydrate Total	21.0g	10.5g
Sugars	16.0g	9.3g
Dietary fibre	0.7g	0.0g
Sodium	<1mg	1.0mg
Oxygen Radial Absorbance Capacity	3200 µmol TE [a]/ g	15.55 µmol TE[a]/ g
Red Pigment (Anthocyanin) content	69 mg [b]	0.02mg [c]

[a] Trolox Equivalent

[b] Iland Method (19).

[c] USDA database for the flavonoid content of selected foods (release 3.1) (14).

Measurements

Outcome variables were measured at three time points: baseline, 6 weeks and 12 weeks with all interviews conducted at the same time of the day (am). At baseline and 12 weeks participants arrived fasting and a blood sample was collected by a trained phlebotomist. A standardised breakfast was offered to participants (cereal, milk, banana, tea and/or coffee) and they were instructed to consume the meal ad-libitum, before the remainder of the interview was conducted. At 6 weeks, participants arrived at the clinic after having consumed their usual breakfast at home.

At each interview, a questionnaire was administered by a single researcher, with the assistance of a guardian or carer, where appropriate, reporting selected demographic characteristics, tobacco use, and consumption of alcohol, supplements and medications. Nutritional status was assessed using an interviewer-administered Mini Nutritional Assessment (MNA) (20) and dietary intake, including flavonoid intake, was assessed using a 24hr dietary recall method, with the assistance of a carer or guardian. Dietary records were entered into the FoodWorks dietary assessment programme to assess nutritional parameters (Xyris Software, Highgate Hill, QLD, Australia, Version 5, 2007). To estimate usual flavonoid intake, dietary records were cross-referenced with the 2013 USDA database for the flavonoid content of selected foods release 3.1 (14). Resting BP and heart rate were measured using an Omron HEM7200 Deluxe Automatic BP Monitor, while seated, in triplicate and averaged. To monitor physical outcomes over 12 weeks, anthropometric measurements included height, weight, and mid arm and mid-calf circumference. Lawton's Instrumental Activities of Daily Living Scale (IADL) (21) determined functional impairment and hand grip strength was assessed using a digital Jamar handgrip dynamometer (Lafayette

Instruments, Indiana, USA). The blood sample assessed changes in markers of inflammation (C-reactive protein (CRP) and Interleukin-6 (IL-6) and plasma vitamin C levels. Blood samples were prepared and stored at -80^{o}C for batch analysis by an independent laboratory where IL6 was measured by High Sensitivity cytokine panel (millipore), CRP was measured by hsCRP (Kamiya), and Vitamin C by an in-house method.

Outcome assessment

Cognitive function was assessed using a battery of seven cognitive tests, including the Rey Auditory Verbal Learning Test (RAVLT) (22, 23) , the self-ordered pointing task (SOPT)(24), the Boston naming test (25), the trail making test (TMT) (26), digit span backwards task (27) and category and letter verbal fluency (28). The RAVLT measured verbal learning and memory by the participant learning a list of words over presentation-test and delayed-test trials. The SOPT is a test of working memory, and the Boston naming test assessed confrontational naming, related to semantic memory. The TMT A and B evaluated executive function and the relative difference between B and A was used as the measure. Digit span backwards task examined short-term memory storage and executive control processes by requiring participants to repeat a series of numbers in the reverse order they were given. Category and letter verbal fluency measured executive function, requiring participants to recite as many words as possible that belonged to the category “animals” or began with the letter “F” and “A”. The geriatric depression scale (GDS) (29) assessed mood state.

Statistical Analysis

Statistical analysis was conducted using SPSS statistical program (V17.0: 2006, SPSS, Inc., Chicago, II, USA). Analysis was completed using the full cohort that completed each of the time points. Continuous data are described as the mean and standard deviation (SD) as appropriate. Non-normally distributed variables were transformed and treated as normally distributed. Baseline differences between the intervention and control group's characteristics and nutrition information were analysed by unpaired t-tests for continuous variables. A 2-way repeated measures ANOVA analysed the effect of time, treatment, and time*treatment interactions for blood pressure measurements at 6 and 12 weeks. Analysis of covariance (ANCOVA) using the baseline data as a covariate was used to analyse the group effect at 6 and 12 weeks for all cognitive tasks, to isolate the effect of the intervention while controlling for group differences at baseline. Repeated measures ANOVA assessed differences in markers of inflammation and vitamin C at baseline and 12 weeks. A p value less than or equal to 0.05 was considered to indicate statistical significance. Eta-squared (η^2) values were calculated to indicate the strength of the main effect. The Cohen's f effect size estimates were characterised as small (0·10), medium (0·25) and large (0·40) (30).

Results

Forty-nine participants (24 female, 25 male) were recruited and seven participants withdrew from the study (2 indefinitely hospitalised, 4 due to study burden and 1 moved away). Participants were randomised into the control group (n=25) and intervention group (n=24) (Figure 1).

Baseline:

No differences between groups were found for age, BMI and anthropometrics, years of education or total flavonoid intake at baseline. Significant differences at baseline were found between groups for measures of malnutrition, activities of daily living, and hand grip strength.

Differences in habitual intake of several flavonoid subclasses was found between the groups, according to a 24h recall administered at baseline (Table 1), with the intervention group consuming more flavonoids than control subjects. Mean total flavonoid intake was estimated as 510mg/day. Black tea (80%) was the most significant dietary source of total flavonoids followed by green tea (7.5%), red wine (4.5%), apples (1.7%) and oranges (1.6%) with their respective fruit juices. Flavonols contributed 5.15% of total flavonoid intake. Dominant sources included black and green tea, onion, broccoli and apples. Flavones contributed the smallest percentage (0.15%) with the major source being parsley. Total flavanone intake provided 2% with major sources including oranges and orange juice, and lemons. Flavon-3-ols contributed 88.1% of total intake, with black tea as the major source and wine and apples contributing somewhat. Anthocyanidins (4.6%) were provided by red wine, red grapes and bananas.

At baseline, the only significant difference between the groups for nutrient intake was for carbohydrates (p=0.023) and caffeine intake (p=0.03), with the intervention group with a higher intake than the control.

Post-intervention:

A trend for improvement in most of the cognitive tasks is evident as shown by the mean difference between baseline and 12 weeks for the intervention group only (Table 3). Analysis of covariance showed significant improvement in cognitive performance in the intervention group only (Figure 2), at 6 and 12 weeks for the Category Verbal Fluency task (p=0.014) , RAVLT total (p=0.014) , RAVLT delayed recall (p=0.005) and RAVLT 20 minute delayed recall (p=<0.001) tasks. The effect sizes for Category Fluency ($\eta^2 = 0{\cdot}711$), RAVLT total ($\eta^2 = 0.713$), RAVLT delayed recall ($\eta^2 = 0.433$) were large and the effect size for RAVLT 20 minute delayed recall ($\eta^2 = 0.242$) was moderate. No significant improvements from baseline were found for cognitive performance tasks in the control group.

Table 3: Mean scores for cognitive performance and mood by group [a]

	Control group			Intervention group		
	Baseline n=25	12 weeks n=21	Mean Difference	Baseline n=24	12 weeks n=21	Mean Difference
Letter Verbal Fluency (executive function)	13.1 ± 7.5	13.1 ± 7.9	0.015 ± 0.4	18.9 ± 11.0	19.0 ± 10.3	0.13 ± 0.7
Category Verbal Fluency (executive function)	8.4 ± 4.5	8.3 ± 4.7	-0.1 ± 0.19	11.9 ± 4.5	13.4 ± 5.1	1.9 ± 0.17
RAVLT total (I-V) (learning and memory)	19.3 ± 9.2	17.5 ± 13.1	-1.8 ± 3.85	25.5 ± 10.6	29.1 ± 11.5	3.9 ± 0.88
RAVLT delayed recall (memory)	1.2 ± 1.9	1.4 ± 2.2	0.2 ± 0.4	2.3 ± 2.6	3.8 ± 2.9	1.6 ± 0.4
RAVLT 20m delayed recall (memory)	0.72 ± 1.2	0.7 ± 1.2	0 ± 0.07	0.6 ± 1.0	2.3 ± 2.6	1.6 ± 1.4
Trail making task [b] (executive functioning)	129.8 ± 81.1	128.6 ± 85.0	-1.2 ± 4.0	125.1 ± 65.0	101.9 ± 67.3	-23.2 ± 2.32
Self-ordered pointing task (working memory)	1.3 ± 1.3	1.6 ± 1.8	0.3 ± 0.5	0.6 ± 1.0	0.6 ± 0.8	0.0 ± -0.2
Digit span backwards task (short-term memory)	2.3 ± 1.1	2.8 ± 1.1	0.5 ± 0	3.0 ± 1.0	3.4 ± 1.2	0.4 ± 0.2
Boston naming task (semantic memory)	33.1 ± 15.5	31.9 ± 13.6	-1.2 ± -1.8	40.6 ± 13.1	40.0 ± 13.6	-0.5 ± 0.5
Geriatric Depression Scale [b] (mood)	7.3 ± 4.7	6.9 ± 3.5	-0.4 ± -1.2	6.9 ± 4.5	6.3 ± 4.3	-0.6 ± -0.2

[a] Data are given as mean ± SD. Baseline refers to measures obtained at the pre-intervention assessment. Final refers to measures obtained during the final week of the intervention. Difference = final score (mean) less baseline score (mean).

[b] A negative score is indicative of improved outcomes. Otherwise, a positive difference in score indicates improved outcomes.

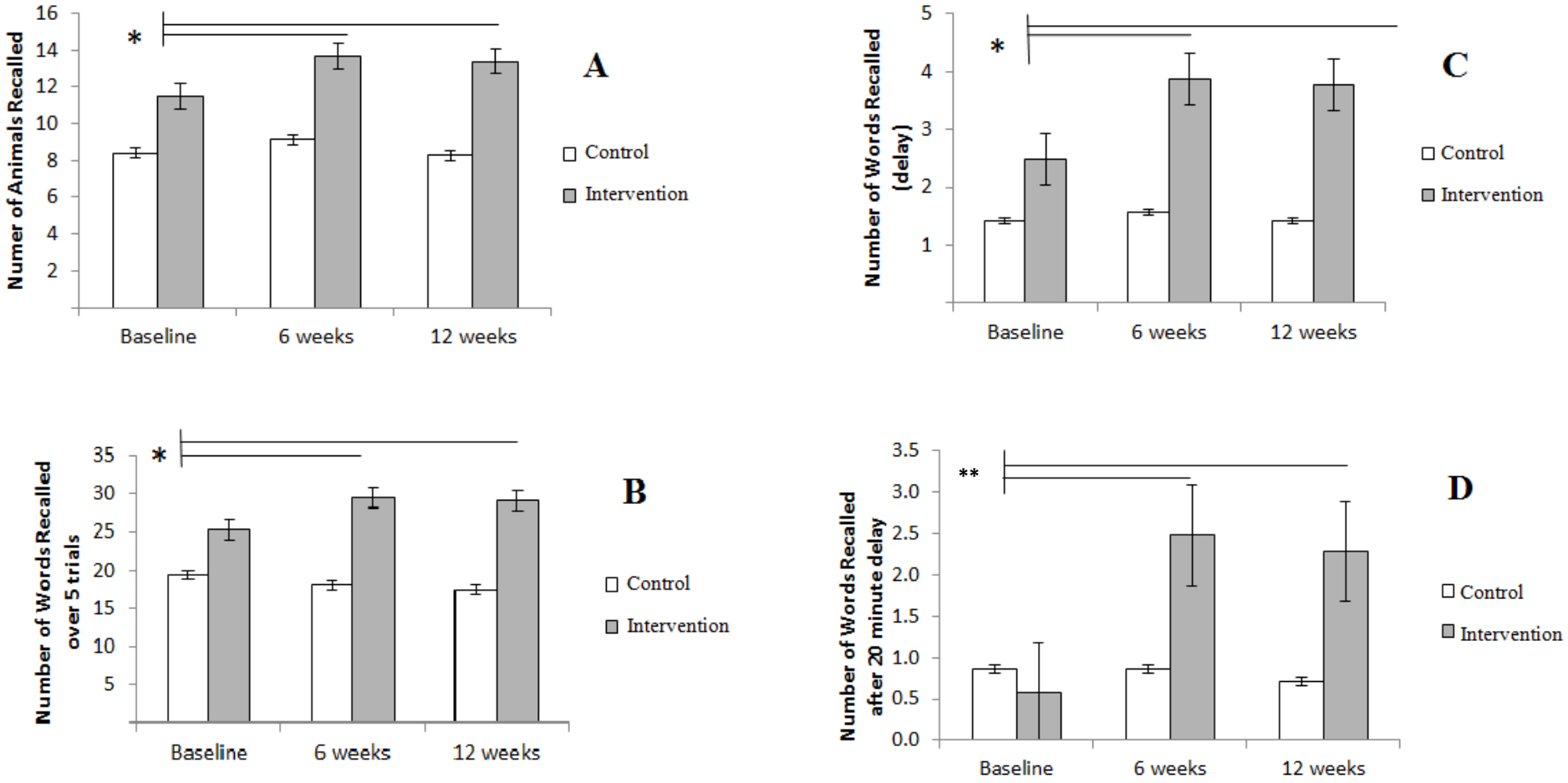

Figure 2: Significant changes in category fluency (A), RAVLT total (B), RAVLT delayed recall (C) and RAVLT – 20minute delayed recall (D) at 6 and 12 weeks post intervention
*p=<0.05**p=<0.001

Table 4: Blood pressure and heart rate measurements according to group at baseline, 6 and 12 weeks [a]

	Control group			Intervention group		
	Baseline n=25	6 weeks n=21	12 weeks n=21	Baseline n=24	6 weeks n=21	12 weeks n=21
Systolic BP*	140 ± 19.7	138.5 ± 12.3	137.0 ± 10.1	138.2 ± 16.4	133.7 ± 9.9	130.5 ± 12.2
Diastolic BP	80.6 ± 9.8	81.0 ± 8.0	81.3 ± 11.6	78.6 ± 11.7	77.0 ± 9.9	77.0 ± 12.6
Heart Rate	70.2 ± 10.2	70.2 ± 11.1	74.2 ± 11.8	67.9 ± 10.7	66.0 ± 7.2	67.5 ± 7.9

*p=<0.05

Data are given as mean (mmHg) ± SD.

[a]Baseline refers to measures obtained at the pre-intervention assessment. 6 weeks and 12 weeks refers to measures obtained at these time points during the intervention.

Repeated measures ANOVA, with the baseline as a covariate, showed a significant difference in systolic blood pressure (p=0.038) at 6 and 12 weeks post baseline, with a similar trend evident for diastolic blood pressure (p=0.160) in the intervention group (Table 4). No significant differences in blood pressure are evident for the control group.

At follow up, there were no significant between-group differences in nutrient intake or for change in nutrient intake from baseline.

Repeated Measures ANOVA showed no significant difference between the intervention and control groups for serum vitamin C, IL-6 or CRP at baseline or 12 weeks (Table 5). There were no improvements in serum vitamin C levels after 12 weeks in either group. Mean levels of plasma CRP and IL-6 were not significantly different after the intervention.

Table 5: Serum vitamin C and inflammatory markers at baseline and 12 weeks

	Control group n=25		**Intervention group** n=24	
	Baseline	12 weeks	Baseline	12 weeks
IL6 (pg/mL)[a]	4.3±2.9	6.3±5.8	3.7±1.8	3.8±3.5
hsCRP (mg/L)[b]	2.0±2.5	2.0±2.3	1.6±1.5	1.7±1.8
Vitamin C (uM)	19.3±13.1	13.6±8.2	18.2±10.6	13.3±11.0

[a]IL-6 Interleukin-6; [b]hsCRP C-Reactive Protein

Data are given as mean ± SD.

Discussion

This study found that daily consumption of a feasible serving of anthocyanin-rich cherry juice for 12 weeks improved cognitive performance across almost all tasks in older adults with mild to moderate dementia. To our knowledge, this is the first controlled human trial examining cognitive and physical responses to a dietary intervention involving sweet cherries as a source of flavonoids and anthocyanins.

Our study suggests that provision of anthocyanins through an achievable and acceptable daily quantity of sweet cherry juice over 12 weeks has benefit for cognitive function in older adults with Alzheimer's type dementia. Trends are evident, showing improvements in cognitive performance across all tasks with regular cherry juice consumption. Statistically significant improvements are seen for category verbal fluency and tasks relating to verbal learning and memory (Figure 2). The moderate and large effect sizes seen for the cognitive tasks highlight the clinical relevance of these cognitive improvements. These findings are consistent with those of recent human and animal studies showing improvement in cognitive performance in these domains with dietary supplementation with other anthocyanin-rich food sources (11-13, 31-33). Although, we have previously shown that intake of cherry juice does not impact acute cognition over 6h (34). The ability of flavonoids to modulate Alzheimer's disease progression is still poorly understood (17). As explored by the hypothesis in the current study, flavonoids may be more likely to hinder both normal and disease-related losses in cognitive performance through their actions on the brain's cellular and molecular architecture of memory, rather than halt disease progression.

Some nutritional differences existed between the intervention and control juices (Table 2). As intended, the ORAC measure of oxidative capacity (3200 vs 15.55 μmol TE/g) and the red

pigment (anthocyanin) content (690mg/L vs 0.2mg/L) is much higher in the experimental cherry juice compared to the control apple juice. Apple juice was chosen as a control juice as it represents a commonly consumed beverage in older adults, but that has negligible anthocyanin. Despite literature indicating beneficial effects of apples and apple flavonoids for health outcomes, the juice utilised in the experiment was processed in a way that likely degraded the flavonoid content. This important aspect of the research design is relevant to translation of the findings into dietary messages. However, it is important for future work to investigate whether dose-response effects are evident in such nutrition interventions.

Despite randomisation, differences in several anthropometric and nutritional factors existed between the intervention and control group at baseline (Table 1). The estimation of total flavonoid intake and intake of specific flavonoid subclasses at baseline revealed large variability between subjects. This can be principally attributed to variations in tea (confirmed by significant differences in caffeine intake between groups), wine and other fruit juice consumption, which were the main sources of flavonoids in this sample. Total intake and major sources of flavonoids of participants at baseline was similar to that reported for Australians aged 65+ years (510 vs 575 vs 683mg/day respectively) (4, 35, 36). The higher anthocyanin intake in our sample, compared to national estimates, may be explained by a relatively high consumption of red wine in the study group. The addition of cherry juice to the diet of the intervention group provided them with an additional 138mg anthocyanin/day, which increased their total anthocyanin intake to 46 times greater than the national estimate for the daily intake of adults aged 65+y (3.02mg/day). In the absence of a Nutrient Reference Value (NRV) for flavonoids (37), this magnitude of increase in consumption can be considered to be a significant increase above habitual intake levels for this age group.

Secondary outcome measures included anti-inflammatory effects, changes in functional and physical ability and depressive symptomatology. There was a significant reduction in systolic blood pressure and a trend for diastolic blood pressure in the intervention group; however the study was not adequately powered to detect blood pressure changes. Previous intervention trials have hypothesised that the mechanisms relating to the improvements seen in cognitive performance after anthocyanin-rich food supplementation are due to a reduction in inflammatory markers, resulting in a blood pressure-lowering effect (13). Our study showed that markers of inflammation were not significantly altered after cherry juice supplementation (Table 5) and remained within the clinically normal ranges (CRP <5mg/mL; IL-6 <10pg/mL). This finding suggests that the bioactive components provided by the cherry juice may provide other benefits, such as stimulating an up-regulation of signalling cascades in areas of the brain relating to memory (8). Additionally, as Alzheimer's disease is associated with progressive and chronic inflammation (38), the disease pathology may mask any potential anti-inflammatory effects provided by the cherry juice.

Surprisingly, serum vitamin C levels decreased in both groups at 12 weeks of follow up (Table 5). This difference cannot be explained through changes in dietary patterns over the study duration as vitamin C intake did not differ according to 24h diet recall data. Additionally, self-reported fruit and vegetable intake was not significantly different at baseline and follow up. A possible explanation may be the study juices may have replaced consumption of commercial fruit juices that could have been fortified with high levels of ascorbic acid. The reported reduction in serum vitamin C parameters is not clinically significant as mean levels remained in the clinically normal range (11-114 micromoles/L). Although under-researched in humans, some pre-clinical evidence indicates that high-doses of flavonoids may inhibit ascorbate absorption (39), which may explain the reduction in

serum vitamin C. Alternatively, the reagents that were utilized to prepare the serum prior to Vitamin C analysis may have degraded over the study period.

The study limitations include a relatively small sample size and short intervention length (12 weeks). However, preclinical studies indicate that flavonoids from berry fruits may require only several weeks to accumulate in brain regions associated with cognition (40). Despite this, a short time frame limits our ability for observations regarding changes in dementia progression. Given the moderate to large effect sizes found in this study, a longer duration of follow up with larger numbers would be the next progression in research. Another limitation of the study relates to the generally better cognitive and physical ability of the intervention group, which is evident despite randomisation. Interestingly, the measure of effect for improvements on cognitive tasks in the intervention group was the same, regardless of the cognitive ability of participants. At the baseline and 12 week visits, a standardised breakfast meal was provided, however at the 6 week visit participants arrived at the clinic facility in a non-fasted state, after having consumed their usual breakfast at home (as a fasting blood sample was not collected). If there was a large difference in the nutritional composition of their usual breakfast to the standardised breakfast (e.g. a significantly greater intake of caffeine), this may have either positively or negatively influenced cognitive performance at this time point. However, this effect was minimised by the participants consuming their breakfast at 6 and 12 weeks ad-libitum, and as the improvements in cognitive performance seen at 6 and 12 weeks are not significantly different, a difference in breakfast is unlikely to be of great importance. Lastly, while it is likely the bioactivity of the cherry juice relates to its high anthocyanin content, the potential bioactive effects of other polyphenols cannot be excluded.

Regardless of their exact mechanisms, the potential of flavonoids to improve cognitive outcomes for older adults with Alzheimer's type dementia cannot be underestimated and a notable strength of this study is the very low possibility of any harm to this vulnerable group. Further research is required to improve the knowledge base to inform dietary recommendations for this patient group, as an adjunct to traditional dementia treatment. For older adults living with reduced cognitive capacity, the inclusion of an anthocyanin-rich beverage, in addition to or in replacement of another processed fruit juice, may be a practical way to improve their total flavonoid consumption, which has been shown to provide positive outcomes for prevention of cognitive decline.

Conclusion

The findings of this study suggest that regular anthocyanin-rich cherry juice consumption over 12 weeks can improve cognition in older adults with dementia and provides a basis for more comprehensive human trials to study the potential of cherry flavonoids to influence neuro-cognitive health.

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