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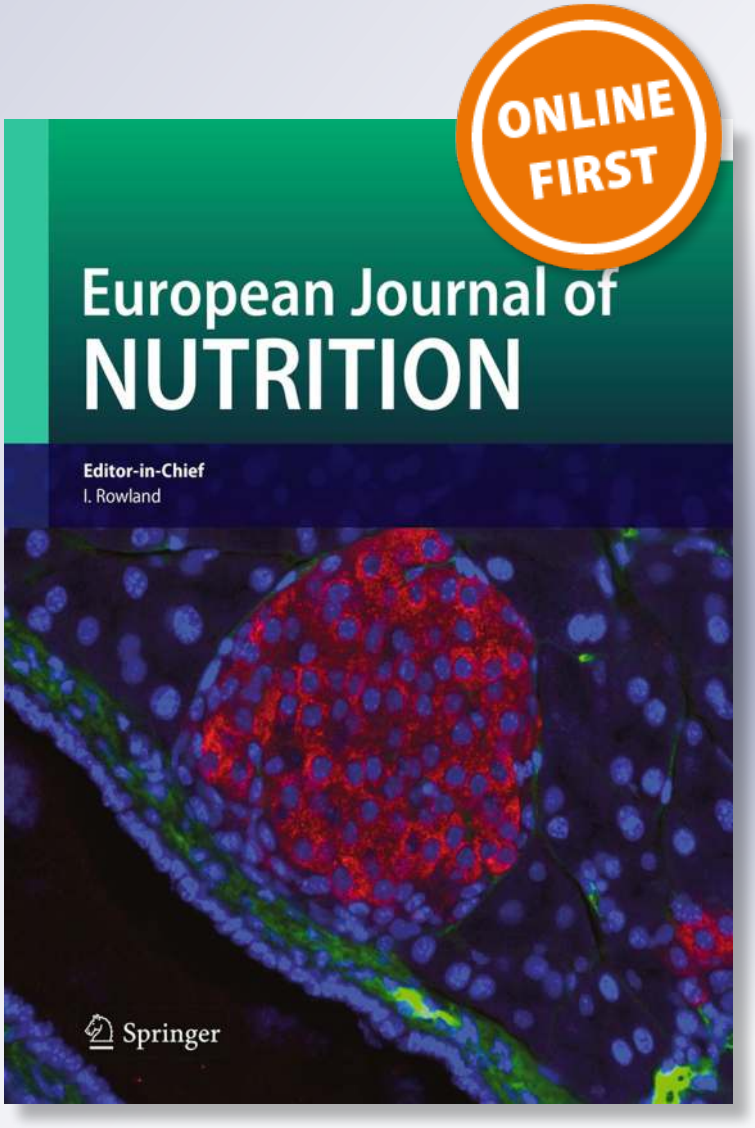

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ORIGINAL CONTRIBUTION

Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children

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Abstract
Purpose Previously, anthocyanin-rich blueberry treatments have shown positive effects on cognition in both animals and human adults. However, little research has considered whether these benefits transfer to children. Here we describe an acute time-course and dose–response investigation considering whether these cognitive benefits extend to children.
Methods Using a double-blind cross-over design, on three occasions children ($n = 21$; 7–10 years) consumed placebo (vehicle) or blueberry drinks containing 15 or 30 g freeze-dried wild blueberry (WBB) powder. A cognitive battery including tests of verbal memory, word recognition, response interference, response inhibition and levels of processing was performed at baseline, and 1.15, 3 and 6 h following treatment.
Results Significant WBB-related improvements included final immediate recall at 1.15 h, delayed word recognition sustained over each period, and accuracy on cognitively demanding incongruent trials in the interference task at 3 h. Importantly, across all measures, cognitive performance improved, consistent with a dose–response model, with the best performance following 30 g WBB and the worst following vehicle.
Conclusion Findings demonstrate WBB-related cognitive improvements in 7- to 10-year-old children. These effects would seem to be particularly sensitive to the cognitive demand of task.

Keywords Flavonoid · Children · Anthocyanin · Cognition · Memory · Executive function

Introduction

It is widely accepted that diet has an influence on the cognitive capabilities and development of children [1]. To date, research has focused primarily on maternal diet during pregnancy, the chronic effects of micronutrients and polyunsaturated fatty acids during childhood, the acute effects of eating occasions (such as breakfast) and different carbohydrate loads [2–4]. Few intervention studies have investigated the acute and chronic effects of specific micro- or macro-nutrients on cognitive performance in school-aged children. However, where this research has been done, benefits have been found on non-verb`al intelligence resulting from multivitamin supplementation [5, 6] and for sustained attention following acute carbohydrate intervention [7, 8] though evidence of beneficial effects following polyunsaturated fatty acids intervention remains equivocal [9]. To our knowledge, however, no acute fully controlled, double-blinded research on the effects of flavonoids, found naturally in foods such as fruit, vegetables, teas and fruit juices, on cognitive behaviour of children has been carried out.

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The health benefits of flavonoids, such as improvements in coronary and vascular function, are well documented [10]. There is also a growing body of research from preclinical and adult human trials indicating that both chronic and acute interventions with flavonoids can lead to cognitive improvements in animals and humans [11–14] with berries (the main source of anthocyanins, a particular class of flavonoids, in the human diet) being known to protect against neuronal stress [15] and positively mediate signalling pathways in the brain [16]. Indeed, preclinical work has found that 7–12 weeks of supplementation with blueberry anthocyanins produces significant improvements in rodent visuo-spatial memory [17, 18]. Similarly, following one-off interventions in adults, improvements have been reported following acute cocoa flavonol interventions on memory-related areas such as spatial working memory [19] and attention-related executive function tasks such as the serial 3 s and RVIP [20]. Chronic supplementation has also shown improvements in visuo-spatial memory following supplementation with pinus radiata extract of proanthocyanidins for 5 weeks or 3 months [21, 22] and immediate verbal memory following 12-week supplementation with blueberry and grape anthocyanins [23, 24]. The mechanisms by which flavonoids exert these actions on cognitive performance are still being elaborated, including evidence which suggests that they may increase cerebral blood flow (CBF) [25–27] as well as modulate the activation status of neuronal receptors, signalling proteins and gene expression [17, 18, 27, 28].

As discussed above, there has been little research investigating flavonoid-related cognitive interventions with children. Currently, only two short reports have been published. Firstly, a study by Calderón-Garcidueñas and colleagues showed that supplementing children (mean age 10.55) with a 680-mg dose of cocoa flavanols for a period of between 9 and 24 days produced marginally significant improved performance on letter and object span tests for 15 of the 18 participants. Importantly, however, this study did not control for the duration of intervention or amount of sugar consumed with each drink [29]. More latterly from our own laboratory, in a within-subjects pilot study testing children aged between 8 and 10 years [30], we found improvements in delayed memory performance following acute intervention with a 200-g fresh high-bush blueberry drink containing 143 mg anthocyanins. Though, on this occasion, we did not find improved performance on executive function tasks, the positive memory findings give a solid basis upon which to expect further cognitive benefits to become evident in a more comprehensive time-course and dose–response study.

Seven- to ten-year-olds were chosen for the present study because, coinciding with a spurt in frontal lobe growth, children of this age have sufficient cognitive ability to competently perform the type of executive function and memory tasks which have shown improvement in adult studies [31–33]. With regard to the tasks, a study by Hillman et al. [34] has shown an interference task, the Modified Flanker Task (MFT), to be sensitive to acute exercise (which induces increases in Brain-Derived Neurotrophic Factor (BDNF) and CBF in a similar way to acute flavonoid interventions) in 9.5 ± 0.5 years children. Furthermore, the MFT and Go–NoGo tasks are known to activate the same brain areas in children (dorsolateral prefrontal cortex, anterior cingulate cortex) [27] which have been found to show increased activation in adult research following a flavonoid intervention [35, 36]. Given that flavonoid intervention has a positive effect on BDNF levels, and also that studies have shown learning and memory effects following flavonoid intervention [25, 26, 30], a modified version of the Rey's Auditory Verbal Learning Task (AVLT) was developed. This task examines performance in both learning and memory recall and has also proven to be sensitive to levels of attention in children aged 8–12 [37]. Finally, in order to ascertain whether flavonoids have an effect on processing speed, a levels-of-processing Picture Matching Task (PMT) [38] was introduced.

Lamport et al. [11] note that, though positive cognitive effects are often found, an association between polyphenol (and therefore flavonoid) dose, duration of intervention and cognitive performance has yet to be established. Given this lack of consistency in the data obtained to date, future work should aim to establish dose, duration and performance effects of flavonoid supplementation on cognition. Such work is of relevance in children and adolescents because the positive cognitive effects, if translated to this age group, would be beneficial in an educational setting. Here, therefore, we describe a dose and time-course study examining the acute effects of blueberries (spp. *Vaccinium angustifolium*; rich in anthocyanins) on children aged 7–10 years completing a range of cognitive tasks.

Methods

This study was reviewed by the University of Reading Research Ethics Committee and was given a favourable ethical opinion for conduct.

Participants

An a priori power analysis (using G Power 3.1.9.2) based on the significant findings from our previous work [30] revealed that 23 participants would be required to achieve a power of 0.8. In order to achieve counter-balancing of the three interventions, 24 participants (14 females) aged 7–10 years of varying ethnicity were recruited from two local schools located in ABC1 areas of the UK. Written

consent was obtained from parents or legal guardians in advance of the child's participation. On initial recruitment, parents or legal guardians confirmed that the children spoke English as a first language, had not been diagnosed with ADHD or dyslexia, and had no known fruit or fruit juice intolerance. Participants completed the children's version of Ravens Coloured Progressive Matrices (RCPM) as a measure of fluid intelligence, and "word definitions" and "verbal similarities" from the British Ability Scales II (BAS II) as measures of crystallised word understanding. Parents or guardians completed the ADHD Rating Scale IV and the Edinburgh Handedness Inventory on behalf of the participants. No participants were extreme outliers on any these measures. Three participants were excluded because they failed to consume at least one of the treatments. Thus, all analyses were completed on data from 21 participants. A post hoc power analysis was therefore conducted calculating power values for all significant treatment-related results reported below. This revealed an average post hoc power of 0.68.

Demographic details of the participants are shown in Table 1.

Treatments

On each test day, participants were administered a drink containing either 15- or 30-g freeze-dried wild blueberries (WBB) or vehicle-only treatment. Each participant completed three treatment days with a 7-day washout between treatments. Our 30-g WBB (equivalent to ~240 g fresh wild blueberries; 108 kcal) treatment contained 253 mg anthocyanins, whilst our 15-g (equivalent to ~120 g fresh wild blueberries) treatment contained 127 mg anthocyanins. Both the vehicle and 15-g WBB treatments had fructose, sucrose and vitamin C added in order to match levels of these nutrients with the 30-g WBB treatment. Prior to each test day, a confederate placed all powders into an opaque drinking cup with a small opening for a straw, and all cups were then labelled with the participant number. Half an hour before intervention all powders were mixed with 30 ml of low-energy fruit squash (Rocks brand, UK: 8.4 kcal) a low polyphenol drink (13.2 mg in total) and 170 ml of water giving a total of ~220 ml in liquid to consume. This was added by the experimenter to the cup through the opening using a funnel and then shaken to fully mix the contents. Treatments were consumed through a black straw by the participant. Both experimenter and participant therefore remained blind to the treatment on each test day. Treatments were administered in a fully counterbalanced order across participants.

Table 1 Mean (SD) values for demographic data

Variable	All participants	Females	Males
n	21	12	9
Age	8.7 (0.67)	8.5 (0.66)	8.9 (0.67)
Ravens	27. 7 (3.25)	27.8 (3.25)	27. 6 (3.43)
Word definitions	111.3 (17.4)	109 (19.4)	114.3 (14.8)
Verbal similarities	100.5 (15.6)	102 (16.5)	99.2 (15.2)
ADHD	9 (8.62)	5.7 (6.61)	13.3 (9.37)
Fruit + veg portions	4.9 (2.32)	5.5 (2.16)	4.1 (2.38)

Cognitive tests

E-Prime V2 (Psychology Software Tools, Inc.) running on a 15-inch Toshiba Satellite laptop was used to display the stimuli and record participant responses. To present the audio stimuli during the AVLT task, and also to control for external noise, participants wore enclosed headphones throughout all tasks.

1. *Auditory Verbal Learning Task* (*AVLT*) This task examined performance in learning, memory recall and recognition. The AVLT consisted of five consecutive free recalls (Recalls 1–5) of the same 15 nouns (list A) presented auditorily at a rate of 1 word per second. A further list of fifteen nouns (list B) was then presented as an interference list and recalled once only (Recall B). There was then a further free recall of list A (Recall 6) followed by a 15-min delay and then a final free recall of list A (Recall 7). Finally, participants were shown a list of 50 nouns, containing all the words from lists A and B plus an additional 20 filler words, and asked to circle only the words from list A. Different lists were created for each test session with all words having AOA ratings of <400 (equivalent to age 7 and below) and being matched (all *p*s $\geq$ .49) for concreteness and familiarity (see Online Resource 1).

For each test session, we calculated the following outcomes as specified in Lezak et al. [39]: immediate word span (Recall 1)—showing immediate free recall ability; number of words learned (Recall 5 minus Recall 1)—showing learning over the session; final acquisition level (Recall 5)—showing the total number of words learned; proactive interference (PI; Recall B minus Recall 1)—indicating the effect of previously encoded words on the encoding of new words; retroactive interference (RI; Recall 5 minus Recall 6)—indicating the effect of encoding new words on previously encoded words and word recognition expressed as the number of correctly circled words.

2. *Modified Flanker Task* (*MFT*) This task examined response interference. Using the method of Hillman et al. [34], arrow symbols "<" and ">" were presented

in white against a black background five in a row. The middle arrow was either congruent (i.e. <<<<< or >>>>>) or incongruent (i.e. <<><< or >><>>) with the pairs of arrows on either side. The stimulus was displayed for 120 ms and was followed by a pseudorandom inter-stimulus interval of 1000, 1300 or 1500 ms. There were 100 trials with presentation randomised so the arrows appeared with equal probability of congruence and direction. Participants were instructed to press the left and right arrow keys on the keyboard according to the direction of the centre arrow.

Accuracy and response times (RTs; with RTs <100 ms removed) for both congruent and incongruent trials were measured separately. Additionally, we calculated interference effect measures separately for accuracy and RT by subtracting incongruent trial performance from congruent trial performance.

3. *Go–NoGo* This task examined response inhibition. Participants were shown stimulus slides of either a cartoon mole (Go target) or a cartoon rabbit (NoGo non-target) which were presented for 300 ms. This was followed by a fixation slide of an empty mole hole presented for 1300 ms. The object of the game was explained as saving the garden by using the right hand to press the space bar in order to "whack" the moles as they popped out of the hole, or to avoid "whacking" the rabbit. There were 100 trials 25 of which were NoGo trials.

A *d*-prime measure was calculated by subtracting the false alarm rate *z*-score from the hit rate *z*-score (both normalised using the Excel "normsinv" function as specified in Stanislaw and Tordorov [40]) for each child. False alarm rate and RTs (Go trials only) were recorded, and a speed/accuracy trade-off was calculated by converting the false alarm rate and RTs into *z*-scores (normalised to SD of overall false alarm rate and RTs, respectively) and then dividing the resulting RT *z*-scores by the false alarm *z*-scores.

4. *Picture Matching Task* (*PMT*) This task investigated both levels of processing and response interference. Line drawings of a banana, book, jack in the box, and umbrella shown in either open or shut states, similar to those used by Bisanz et al. [38] were created giving eight pictures in total. As can be seen in Fig. 1, combinations of pairs of items were shown to participants which were either Physically Different and Name Different (PDND), Physically Different and Name Same (PDNS), or Physically Same and Name Same (PSNS). Participants performed two versions of the task: in the physical-match task, participants responded yes (by pressing a green key) or no (by pressing a red key) to the pictures according to whether or not they were an exact match (i.e. a yes response should be made in the PSNS condition), whereas in the name-match task, participants were instructed to respond yes or no according to whether or not the pictures had the same name (i.e. a yes response should be made for both the PDNS and PSNS conditions). Participants were shown the picture pairs on either side of a fixation cross which remain displayed until the participant responded. Trial intervals were filled with a 200-ms fixation cross.

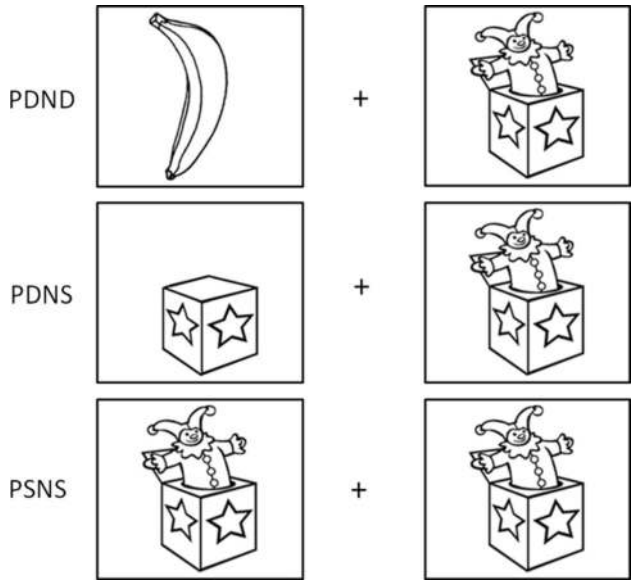

Fig. 1 Example of pairs of stimuli as used in the PMT. Physically Different/Name Different (PDND) should always receive a "no" in both the physical-match task and name-match task. Physically Different/Name Same (PDNS) should receive a "no" response in the physical-match task and a "yes" response in the name-match task. Physically Same/Name Same (PSNS) should always receive a "yes" response in both the physical-match task and the name-match task

Median reaction time (with RTs <100 ms removed) and accuracy were measured for all PDND, PDNS and PSNS trials.

Procedure

Pretest session

On the evening before the first test session, a pretest session took place at the participants' home address where the RCPM, word definitions and verbal similarities from the BAS II, the ADHD Rating Scale IV and the Edinburgh Handedness Inventory were administered. Participants also completed 11 practice trials of the Go–NoGo, 12 of the MFT and 16 trials each of the physical and named conditions of the PMT in order to become accustomed

to these tasks prior to the test days. Where participants showed <50 % accuracy on a task further trials were completed until the participant achieved at least 50 % accuracy. All participants achieved >50 % accuracy for the first practice of the Go–NoGo (mean = 75 %) and PMT task (physical-match task mean = 85.4 %; name-match task mean = 88.5 %) conditions. The MFT was found to be more demanding with two children needing two attempts, eight children needing three attempts, one child needing six attempts and one child needing nine attempts before achieving >50 % accuracy. Parents or guardians of the participants were provided with a list of foods to avoid in order to ensure a low-flavonoid diet was consumed on the evening before each test session.

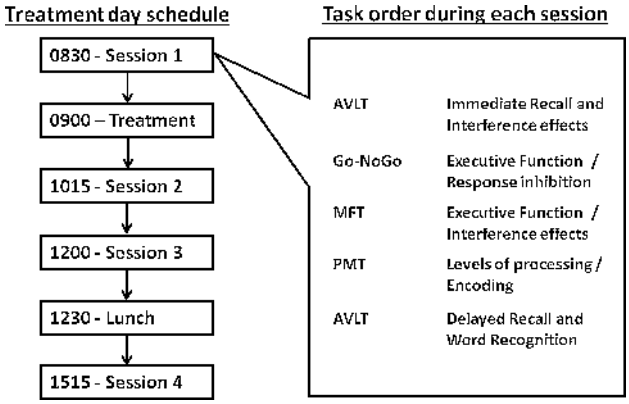

Fig. 2 Schedule of sessions, intervention and task order for each test day

Treatment days

On each test day, a low-flavonoid breakfast, snacks and lunch were provided. The breakfast consisted of 30 g cornflakes with 125 ml of semi-skimmed milk and 5 g of sugar for breakfast (254 kcal in total). The mid morning snack was a small (125 g) banana (~80 kcal) and lunch consisted of a 25-g bag of salted potato crisps and a plain sandwich containing two slices of white sliced bread, 10 g of butter and either 26 g ham, 20 g chicken or 22.5 g cheese as a filling (matched for energy content—404 kcal in total). Participants drank only water during the test day and were allowed to consume as much as they wished. Participants were tested in quiet unoccupied rooms in the two schools from which the participants were drawn. On each treatment day, following breakfast, an initial baseline test session took place at 0830 hours (test session 1). The treatment was then consumed at 0900 hours with the participants being given 10 min to consume the drink in its entirety. Three further test sessions took place (±10 min) at 1015 hours (test session 2), 1200 hours (test session 3) and 1515 hours (test session 4). The snack and lunch were consumed immediately after the second and third test sessions, respectively. During each test session, participants completed the tasks in the order: AVLT Recalls 1–6, MFT, Go–NoGo, PMT, AVLT Recall 7 and Word Recognition (see Fig. 2).

Following the final test session, participants were debriefed regarding the purpose of the study and informed of the contents of the drinks. All children received a £10 book token though this payment was not disclosed until completion of the study.

Analysis

To confirm that performance was broadly consistent with previous studies, an initial non-change from baseline analysis was conducted where appropriate using raw behavioural data. Additionally, we compared the pre-intervention baseline data for each condition on each test day to ensure that there were no significant differences in baseline performance. For all test conditions, comparisons between baseline performance on each test day were non-significant (all *p*s > .082). All post-intervention measures for AVLT and Go–NoGo and MFT interference effect were then analysed as change from baseline using 3 × 3 (Treatment × Session) repeated-measures ANOVAs. In order to further explore dose dependency, linear and quadratic contrasts were performed for treatment in the above ANOVAs. Dependent variables (DV) for the MFT were analysed separately as change from baseline using 3 × 2 × 3 (Treatment × Congruency × Session) repeated-measures ANOVAs. Dependent variables for the PMT name-match and physical-match tasks were analysed separately as change from baseline using 3 × 3 × 3 (Treatment × Picture Type × Session). Again, linear and quadratic contrasts were performed for treatment effects in the above ANOVAs. Bonferroni adjustment for multiple comparisons was used for all post hoc analyses. Finally, to analyse the composite effects of anthocyanin dose on overall cognitive performance across the battery, all change from baseline measures for all participants at each time point were analysed by a Page's test [41] for monotonic ordered treatment effects.

SPSS v 19-21 was used to carry out the analyses.

Results

Auditory Verbal Learning Task

As shown in Fig. 3, during each test session, participants recalled a greater number of words from the primary word list on each subsequent attempt of the first five recalls (shown as Rec1–5) indicating a significant learning effect, $F(2,80) = 107.3$, $p < .001$, $\eta_p^2 = .843$. Additionally, as

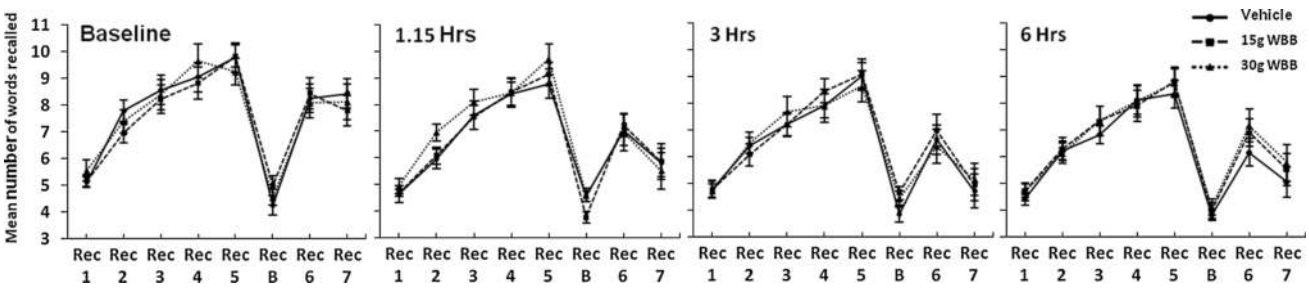

Fig. 3 Word recall performance on the AVLT for the baseline session and three post-intervention sessions at 1.15, 3 and 6 h. Maximum score for each recall is 15. Mean Immediate and Delayed Recall (±SE of the mean) performance for the AVLT is shown for each recall attempt by vehicle, 15 and 30 g WBB. Performance shows the expected learning and within-session retroactive interference effects (as shown in the poorer performance for recall 6) and between session proactive interference effects (as shown in a general decline in words recalled on each subsequent session)

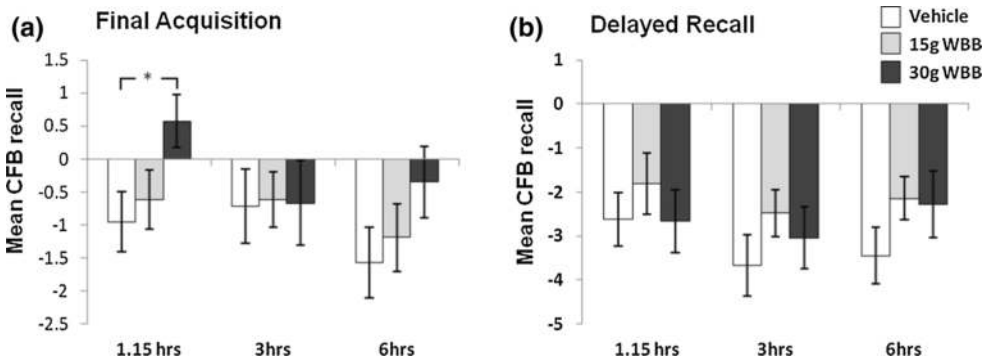

Fig. 4 Mean change from baseline recall (± standard error of the mean) for **a** final acquisition (recall 5), and **b** Delayed Recall performance at all time points following vehicle, 15- or 30-g WBB intervention. All scores calculated by subtracting number of words recalled at baseline (max 15) from number of words recalled at each subsequent post-intervention session. Final acquisition performance can be seen to significantly improve (*$p < .05$ following Bonferroni correction) at 1.15 h following 15-g intervention in comparison with vehicle. There is evidence of a less steep decline in Delayed Recall performance at all time points following 15- or 30-g intervention in comparison with vehicle; however, this fails to reach significance

we would expect, there was also evidence of cumulative, between list, proactive interference (PI) over the course of each test day, with participants, irrespective of treatment, showing a decrease in the average number of words recalled in each subsequent test session, $F(3,60) = 14.8$, $p < .001$, $\eta_p^2 = .426$.

As can be seen in Fig. 4a, participants showed a reduction in final acquisition for all time points throughout the day with the exception of 1.5 h following intervention where the 30-g WBB intervention showed an increase from baseline of 0.57 words. A Drink × Session ANOVA suggested a weak trend for session, $F(2,40) = 2.56$, $p = .09$, $\eta_p^2 = .113$, along with a significant simple main effect of treatment also being evident 1.15 h following intervention, $F(2,40) = 4.13$, $p = .023$, $\eta_p^2 = .171$. A significant positive linear trend was also found for treatment at 1.15 h where, as can be seen from Fig. 4a, the vehicle shows the greatest negative change (mean = −.95) and 30 g WBB the greatest positive change (mean = .57), $F(1,20) = 8.76$, $p = .008$, $\eta_p^2 = .305$. Post hoc analysis of this main effect suggests that the improvement in the 30-g WBB condition contrasted significantly with the vehicle which showed a decrease in recall of 0.95 words at this time point, $p = .023$, following Bonferroni correction for three comparisons. However, no significant difference from vehicle was found for the 15-g WBB treatment which showed a decrease in recall of 0.6 words. The effect seen at 1.15 h was not maintained over the remaining test sessions.

For all treatments, there was a significant decrease in participants' Delayed Recall (Rec7 in Fig. 3) at 1.15, 3 and 6 h after the baseline session, $F(3,60) = 23.5$, $p < .001$, $\eta_p^2 = .541$. However, for the 15-g WBB treatment, this decrease was smaller in magnitude than both the vehicle and 30-g WBB treatments; indeed, our change from baseline analysis (Fig. 4b) shows a trend towards significantly different performance between our vehicle and 15-g WBB treatments indicating less negative effect on Delayed Recall, $F(1,20) = 3.46$, $p = .078$, $\eta_p^2 = .147$. No significant linear or quadratic trend effects were found for the change from baseline analysis on this measure.

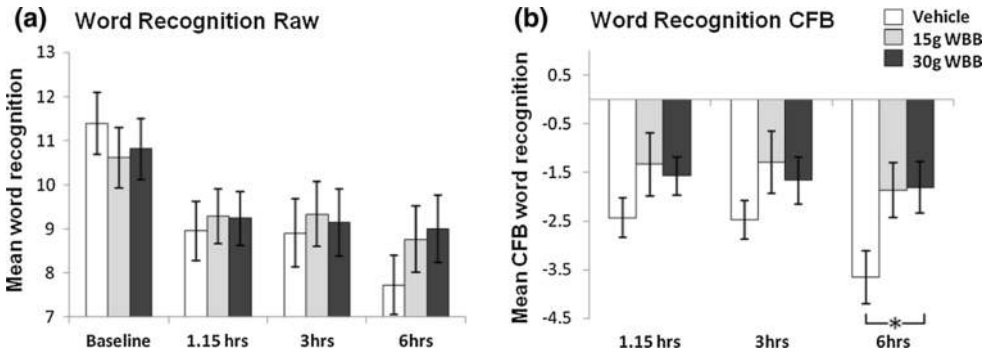

Fig. 5 AVLT word recognition performance. **a** Raw mean word recognition with a maximum score of 15 (±SE of the mean) showing, for each dose, a decrease in performance for the three post-baseline sessions which is particularly marked for the vehicle. **b** Mean change from baseline word recognition (±SE of the mean) indicating a greater reduction in performance at all time points following vehicle in relation to the 15- and 30-g doses which reaches significance at 6 h following intervention (*$p < .05$ following Bonferroni correction). Scores calculated by subtracting number of words recalled at baseline (max 15) from number of words recalled at each subsequent post-intervention session

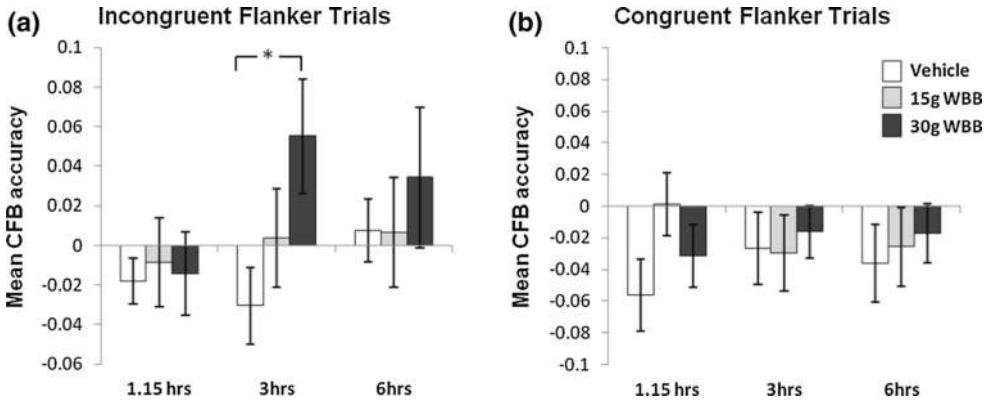

Fig. 6 Change from baseline accuracy for **a** incongruent and **b** congruent flanker task trials (±SE of the mean) showing improved accuracy performance (*$p < .05$ following Bonferroni correction) on incongruent trials at 3 h following intervention in relation to the control drink. Scores calculated by subtracting average accuracy score (max 1) from accuracy score at each subsequent post-intervention session

No significant effects were found for the PI and RI measures.

For all treatments, there was a decrease in Word Recognition at 1.15, 3 and 6 h following the baseline session, $F(3,60) = 23.3$, $p < .001$, $\eta_p^2 = .538$ (Fig. 5a). There was a significant change from baseline main effect of treatment, $F(2,40) = 3.94$, $p = .027$, $\eta_p^2 = .164$, with the vehicle showing a greater decrease in the number of words recognised in comparison with both the 15- and 30-g WBB treatments. A significant positive linear trend was also found for treatment, $F(1,20) = 6.86$, $p = .016$, $\eta_p^2 = .255$. Subsequent analysis found that the decrease in performance for the vehicle drink was significantly different to the decrease seen for both the 15-g WBB, $F(1,20) = 6.57$, $p = .019$, $\eta_p^2 = .247$, and 30-g WBB treatments, $F(1,20) = 6.86$, $p = .016$, $\eta_p^2 = .255$. As can be seen in Fig. 5b, the vehicle treatment showed the poorest change from baseline recognition performance 6 h post-intervention where a decrease of 3.66 words could be seen. In comparison, the 15-g WBB treatment showed a decrease of only 1.85 words, $p = .038$ following Bonferroni correction for three comparisons, and the 30-g treatment showed a decrease of only 1.81 words; however, this failed to reach significance, $p = .131$ following Bonferroni correction for three comparisons.

Modified Flanker Task

As expected, our analyses revealed that response interference was evident with participants showing significantly lower accuracy, $F(1,20) = 1.83$, $p < .001$, $\eta_p^2 = .522$, and slower response times, $F(1,20) = 22.8$, $p < .001$, $\eta_p^2 = .532$ for the incongruent trials in relation to the congruent trials. Furthermore, change from baseline analysis for accuracy also found a significant effect of congruence for all treatments, $F(1,20) = 4.59$, $p = .045$, $\eta_p^2 = .522$.

Data were then analysed separately for both the congruent and incongruent conditions with only incongruent trials showing a significant effect of session, $F(2,40) = 3.39$, $p = .044$, $\eta_p^2 = .145$. As can be seen in Fig. 6a, there were modest dips in accuracy on incongruent trials at 1.15 h for all treatments, and although this was maintained at 3 h for the vehicle treatment (mean = −0.03),

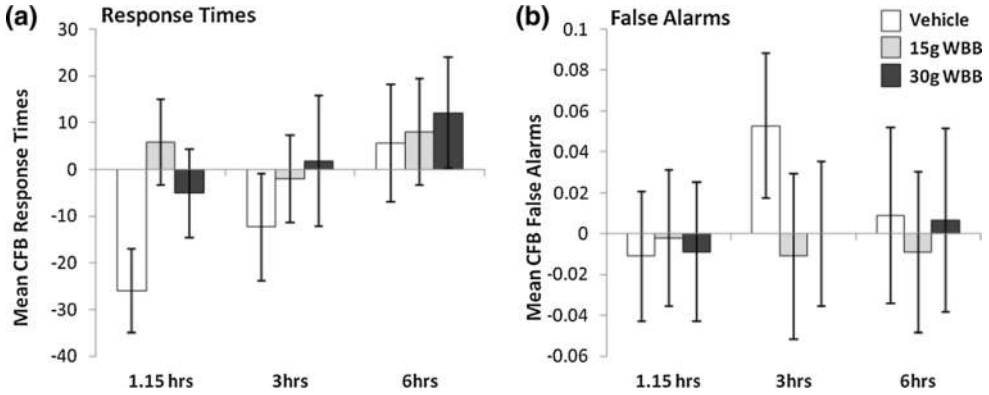

Fig. 7 Change from baseline for **a** Go trial response time (scores calculated by subtracting baseline average response time from average response time at each subsequent post-intervention session) shows a trend for faster response times at 1.15 h for the control intervention in comparison with the 15-g WBB dose, and **b** number of false alarms (scores calculated by subtracting baseline average false alarm score (max 1) from average false alarm score at each subsequent post-intervention session) shows a non-significant increase in false alarms at 3 h for the control intervention

this contrasted with an improvement for the 30-g WBB treatment (mean = 0.055). Importantly, incongruent condition performance between all treatments was found to be significant at the 3-h point, $F(2,40) = 4.02$, $p = .026$, $\eta_p^2 = .167$. Furthermore, a significant positive linear trend was also found for treatment in the incongruent conditions, $F(1,20) = 4.45$, $p = .012$, $\eta_p^2 = .277$. As can be seen in Figs. 3 and 7a, participants performed least accurately following vehicle and most accurately following 30 g WBB at the 3-h point. Post hoc analysis also revealed a significant difference between vehicle and 30-g WBB treatments at the 3-h point, $p = .035$ following Bonferroni correction for three comparisons. Performance for the vehicle treatment returned to levels similar to baseline at 6 h, and though the 30-g WBB treatment performance remained elevated, no statistically significant differences were found. No further significant effects were found for this task.

Go–NoGo Task

Regardless of treatment, change from baseline performance decreased significantly over each subsequent session for Go trial accuracy, $F(2,34) = 3.73$, $p = .034$, $\eta_p^2 = .180$, Go trial RT, $F(2,34) = 4.48$, $p = .014$, $\eta_p^2 = .222$, and also showed a similar trend for the *d*-prime measure, $F(2,34) = 3.04$, $p = .074$, $\eta_p^2 = .142$. Change from baseline comparisons between treatments, however, failed to reach significance for *d*-prime or Go trial accuracy. For Go trial RT, there was a significant effect of treatment at the 1.15-h point, $F(2,34) = 3.59$, $p = .039$, $\eta_p^2 = .174$. As can be seen from Figs. 3 and 8a, the vehicle treatment performance improved by 26.0 ms compared to a modest slowing of 5 ms for the 127-mg treatment ($p = .078$ following Bonferroni correction for three comparisons). No significant linear, $F(1,17) = 3.64$, $p = .074$, $\eta_p^2 = .176$, or quadratic, $F(1,17) = 3.55$, $p = .077$, $\eta_p^2 = .173$, RT trends were found for treatment at the 1.15-h point. No significant main effects were found at 3 or 6 h. All analyses for the accuracy and response time trade-off and false alarm measures failed to reach significance.

Picture Matching Task

As expected, analysis of the raw data revealed that additional processing time was required for the named picture task with participants showing significantly slower response times in comparison with the physical picture task, $F(1,20) = 40.6$, $p < .001$, $\eta_p^2 = .670$. A main effect of session also revealed faster reaction times as the participants progressed through the four sessions $F(3,60) = 10.6$, $p < .001$, $\eta_p^2 = .347$.

Figure 8a and b shows the change from baseline reduction in median reaction time separately for the physical-match and named-match tasks over sessions 2–4. Change from baseline analysis was performed separately on the name-match and physical-match tasks as 3 × 3 × 3 (Treatment × Session × Picture Type) ANOVAs. No significant results were found for the physical-match conditions; however, where the task was cognitively more challenging in the name-match conditions, a significant main effect of picture type was found, $F(2,40) = 4.77$, $p = .014$, $\eta_p^2 = .193$. Though the main effect of treatment was non-significant, $F(1,20) = 2.461$, $p = .098$, $\eta_p^2 = .230$, a significant linear trend was found for this measure $F(1,20) = 5.96$, $p = .024$, $\eta_p^2 = .230$ where, as can be seen from Fig. 8b, for all time points the greatest improvements were found for 30 g

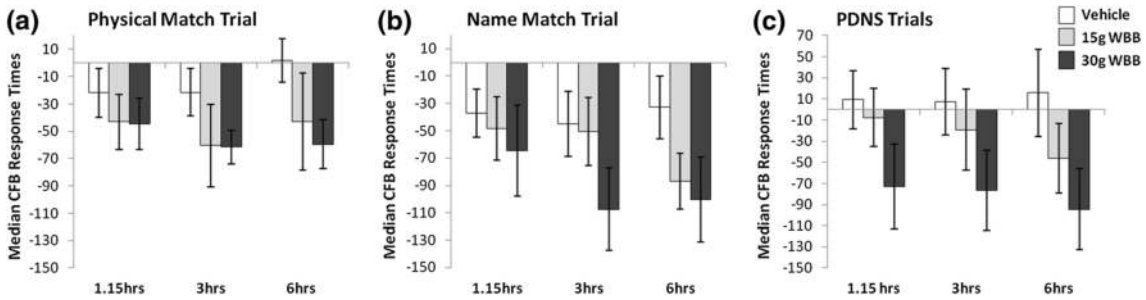

Fig. 8 Change from baseline for median reaction time scores for **a** the physical-match and **b** name-match tasks (±SE of the mean) by treatment and session showing improved change from baseline performance for both the 15- and 30-g intervention drinks for all sessions in comparison with vehicle. **c** Change from baseline for median reaction time scores for the PDNS trials of the name-match task by treatment and session (±SE of the mean) showing improved change from baseline performance for the 30-g intervention in comparison with the vehicle and 15-g treatments. Scores calculated by subtracting baseline median response time from median response time at each subsequent post-intervention session

WBB and the smallest for vehicle treatment. Subsequent analysis of the different picture types revealed no significant effects in the PSNS and PDND conditions; however, the PDNS analysis indicated a trend for a main effect of treatment, $F(2,40) = 2.98$, $p = .062$, $\eta_p^2 = .130$. A significant linear trend was also found for treatment on this measure, $F(1,20) = 6.34$, $p = .020$, $\eta_p^2 = .241$, where it can be seen from Fig. 8c that the greatest improvement in reaction times is found for 30 g WBB.

Analysis of dose effects

As discussed above, there is at present a lack of data concerning dose effects on performance in individual tests [11]; a critical issue in the exploratory analysis of nutritional interventions on cognition is the extent to which a dose–response effect can be generally observed. In the individual analyses conducted above, although not all tests find specific significant results, there often appears to be a dose–response, in which for any given DV, children in the vehicle treatment perform less well than in the 15-g WBB treatment who in turn perform less well than in the 30-g WBB treatment. This observation is further supported by the significant linear trends reported above. Indeed, though the omnibus ANOVA may not have revealed a significant effect, as in the case of the picture recognition task, the subsequent linear trend analysis did show a significant effect with vehicle performance showing the smallest decrease in RT and 30-g WBB performance showing the greatest. As an unbiased test of this possibility, we conducted a nonparametric test of trend [41] on the change from baseline data for each test session and participant (data were laid out as three columns, with each line the performance of one child in one test: see Online Resource 2). Importantly, this test revealed a significant monotonic increase in cognitive performance with anthocyanin dose, $L = 11{,}225$, $p = .009$.

Discussion

The general health benefits of berries are well documented with studies providing support for their neuroprotective, antioxidant, anticancer and anti-inflammatory properties, effects that are ascribed to their high levels of phenolic compounds of which flavonoids are a primary class [51]. A number of animal and adult studies have recently demonstrated positive cognitive effects following acute and chronic [15–18, 24, 30] blueberry interventions; however of these studies, only one has investigated the effects of berries on children [30]. To our knowledge, therefore, the data presented here are the first fully controlled multi-dose, time-course study which demonstrates that acute cognitive benefits can be observed in 7- to 10-year-old children with an anthocyanin-rich blueberry intervention. The Page's test reveals the consistency and strength of this finding with WBB supplementation leading to significant overall improvements in cognition function, with the best change from baseline performance associated with 30-g WBB treatment, intermediate performance with the 15-g WBB treatment and least effective performance with the vehicle treatment. This finding is important because, even when analysed separately, our tasks typically showed a linear relationship between cognitive benefits and increasing WBB dose. The next step in the investigation is clearly to determine more precisely the cognitive locus, and physiological basis, of this effect. Our detailed analyses provide some possible directions for this enquiry.

Analysis of individual cognitive tasks showed that supplementation with anthocyanins produced significant improvements in word acquisition and word recognition, as well as a greater ability to overcome response interference effects as demonstrated in the MFT. These findings give a fuller understanding of the areas where specific benefits in cognition are strongest and may also indicate potential mechanisms

driving the effects. Considering the benefits for memory, we have shown that, when compared to the vehicle treatment, participants demonstrated a significant improvement in final acquisition of a repeated list of words following intervention with 30 g WBB at 1.15 h. This suggests that blueberry intervention had a positive effect on learning at this time point. Additionally, whilst word recognition performance was progressively worse for all doses on each subsequent session throughout the day, this attenuation was significantly less marked for both WBB doses compared with vehicle and suggests a positive effect on secondary (delayed) memory performance. These findings echo our previous research where delayed memory was seen to improve following a fresh high-bush blueberry interventions [30] and are also consistent with Krikorian et al. [24] who found improved performance on word list recall following a 12-week intervention on older adults with wild blueberry juice].

The delayed word recognition effects observed in this study suggest that 30 g WBB and 15 g WBB are effective in maintaining delayed memory performance throughout our 6-h test period. Previous in vivo and in vitro research has found that chronic and acute flavonoid interventions positively influence the ERK-CREB-BDNF signalling pathway related to memory formation in both young and ageing rats [17, 18, 28, 42, 43]. Furthermore, Dodd [27] found that acute supplementation with blueberry anthocyanins maintained BDNF plasma levels in adults in contrast to a reduction following vehicle. BDNF is critical for the formation of short- and long-term memories and learning [44, 45], and there is also evidence to suggest that BDNF contributes most strongly at the point of encoding during recognition tasks [46]. Our findings would seem to be consistent with the maintenance or up-regulation of BDNF levels found following anthocyanin intervention, leading to the facilitation of stronger encoding. Alongside the delayed memory effects seen on the AVLT, children showed greater acquisition of the word lists at 1.15 h following WBB supplementation which may also be linked to elevated levels of BDNF. One alternative explanation for this improvement is that it was driven by a peak in CBF (known to be between 1- and 3-h post-flavonoid intervention in adults [27]) which in turn may have facilitated increased glucose- or oxygen-driven attention at the point of encoding (see [47] for discussion). Furthermore, Rodriguez-Mateos et al. [48] have shown specific increases in endothelium-dependent vasodilation and availability of anthocyanin metabolites at 1–2 and 6 h but not 3 h after blueberry anthocyanin intervention in healthy men. As these are the time periods where we have found the most significant improvements in memory, this gives further support to the possibility and that improvements may be driven by increased blood flow.

The actions of our WBB were not just restricted to beneficial actions on memory-related processing. Participants also demonstrated improved accuracy on the more cognitively demanding trials of the MFT at 3 h following intervention with the 30-g WBB dose. This indicates a possible beneficial effect of WBB on overcoming the interfering effects of distracting non-target stimuli. No significant effects were found on the cognitively less demanding congruent trials, where it is probable that all children, regardless of treatment, were able to perform at a high level, thus giving little room for an improvement to be seen. Similar effects were also noted for the PMT reaction time where no effects were found for the less cognitively demanding physical comparison condition. In the more cognitively demanding name comparison condition, a significant linear trend was evident for RT. Subsequent analysis revealed that this was primarily driven by performance on the PDNS trials where participants overcame interference created by presenting items with the same name but shown as different pictures. An emerging pattern from the data for these two tasks would therefore seem to be that improvement is most likely to be found where tasks (or elements of tasks) are sufficiently sensitive to cognitively challenge the child whilst also allowing room for improvement in performance. To our knowledge, this is the first berry-related intervention to show an improvement in executive function-related performance in children and therefore requires further investigation to confirm and expand these effects with particular reference to the possible sensitivity to cognitive demand.

Although significant attempts were made to control all aspects of this study, a number of potential elements which may have influenced outcomes must be considered. Firstly, the effect of some participants choosing not to consume the intervention resulted in a reduction in power from the anticipated 0.8–0.68. There is therefore an increased probability of type II error in the above analysis. Though the combined Page's test measure goes some way towards demonstrating a global cognitive effect following our treatment, further research is recommended with a larger sample size to confirm and expand on our task specific analysis above. Secondly, though participants were asked for their opinion on drink palatability, no formal investigation was carried out in relation to how participants perceived the drinks to differ in palatability. Previous research has found that palatability can have an effect on mood [49], and it has been proposed that this in turn might enhance cognitive performance [50]. Furthermore, consuming palatable food may also lead to an increased glycemic response in comparison with a matched unpalatable control [51] which may in turn have an effect on cognitive performance. It is therefore important that future studies should consider alternative formulations or capsulation in order to improve the palatability and similarity of intervention drinks. A further factor is that testing took place in a school setting in different classrooms, which meant

some unavoidable amount of variable noise distraction, particularly during the lunch hour. Though this has the benefit of being a more ecologically valid environment, further research in more a controlled environment, along with blood measures of flavonoid and metabolite content, would be useful to confirm and extend our preliminary results. Finally, even though a low-flavonoid diet was consumed the day before, and during, each test day, the children who participated in this study reported a relatively high intake of fruit and vegetables in their normal diet which may have reduced the effects of the intervention. It would be interesting to investigate the action of our blueberry intervention in low fruit and vegetable consumers to see whether more marked effects on cognitive performance would become evident.

Despite these caveats, we conclude that acute blueberry supplementation has a measurable broad, dose-related effect on the performance of 7- to 10-year-old children in a wide variety of cognitive tasks.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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