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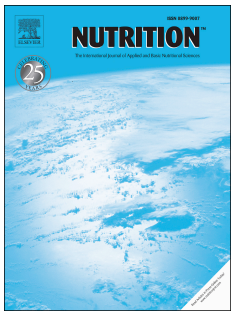

A pilot study investigating the effects of a single dose of a flavonoid-rich blueberry drink on memory in 8-10 year old children

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A pilot study investigating the effects of a single dose of a flavonoid-rich blueberry drink on memory in 8-10 year old children.

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Abstract

Recent evidence from animal and adult human subjects has demonstrated potential benefits to cognition from flavonoid supplementation. This study aimed to investigate whether these cognitive benefits extended to a sample of school-aged children. Using a cross-over design, with a wash out of at least seven days between drinks, fourteen 8-10 year old children consumed either a flavonoid-rich blueberry drink or matched vehicle. Two hours after consumption, subjects completed a battery of five cognitive tests comprising the Go-NoGo, Stroop, Rey's Auditory Verbal Learning Task, Object Location Task, and a Visual N-back. In comparison to vehicle, the blueberry drink produced significant improvements in the delayed recall of a previously learned list of words, showing for the first time a cognitive benefit for acute flavonoid intervention in children. However, performance on a measure of proactive interference indicated that the blueberry intervention led to a greater negative impact of previously memorised words on the encoding of a set of new words. There was no benefit of our blueberry intervention for measures of attention, response inhibition or visuo-spatial memory. While findings are mixed, the improvements in delayed recall found in this pilot study suggest that, following acute flavonoid-rich blueberry interventions, school aged children encode memory items more effectively.

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Introduction

Flavonoids are a class of polyphenols found in abundance in the human diet. There are six main subclasses of flavonoids: flavonols, flavones, isoflavones, flavanones, flavanols and anthocyanidins, and these can be found in large concentrations in foods and supplements such as grapes, blueberries, tea, chocolate, and bark extracts such as Ginkgo biloba. In recent years there has been considerable focus on the health benefits of consuming a diet, or dietary supplement, rich in flavonoids. Animal and human studies have found evidence of benefits to both vascular and cognitive function following flavonoid intervention [1,2,3,4]. For example, chronic supplementation with both young and aging rodents has shown improved visuo-spatial memory in T, radial and Morris water maze tasks (e.g., [5,6]) and improved long-term memory in an inhibitory avoidance task [7].

The improvements in memory seen in rodents are mirrored in adult studies with a recent review by Lamport [4] reporting that positive visuo-spatial memory effects have been found in a number of adult studies (e.g., [8,9]). Lamport also notes that immediate verbal memory would seem to be particularly sensitive to chronic flavonoid or polyphenol berry fruit juice intervention in adults [e.g., 10,11]. Furthermore, although not equivocal, positive results have been found on response interference tasks such as the Stroop task following chronic intervention [12]. Benefits have also been found following acute flavonoid interventions with studies finding improvements in visuospatial memory [13], and also in tasks requiring elevated attention**,** inhibition and executive function [13,14].

A number of mechanisms of action have been proposed to explain the beneficial actions of flavonoids on human cognition, these include facilitating increases in cerebral blood flow (CBF), protecting against neuronal stress, and positively mediating signalling pathways in the brain [2,6,15,16]. Here, research indicates that peak increases in CBF occur two hrs after acute cocoa flavonol intervention [15] while specific increases in endothelium-dependent vasodilation and

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availability of anthocyanin metabolites can be seen at 1-2 and 6hrs following acute blueberry anthocyanin intervention [17].

Whilst it is widely accepted that diet influences the cognitive capabilities and development of children [18], to date no direct research has been reported on whether the cognitive benefits of flavonoid supplementation extend to children. However, were they to be mirrored in a child sample, it could be argued that the effects on memory and attention following flavonoid supplementation would be of benefit in an education setting. Coinciding with a spurt in frontal lobe growth, children between the ages of 7 – 10 develop sufficient cognitive ability to competently perform the type of executive function and memory tasks which have shown improvement in adult studies [19]. Here, therefore, we describe a pilot study investigating the effects of an acute one-off dose of a flavonoid-rich blueberry drink on memory and attention in 8-10 years old children.

Material and Methods

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

Participants

An opportunity sample of 16 year four children (age range 8.08 – 9.83 yrs) were recruited from two primary schools local to Reading University. Participants were screened in advance for fruit allergies, dyslexia and ADHD. As a further control, all participants performed a computerised version of the Raven's Progressive Matrices (RPM) to give a measure of fluid intelligence. Here, participants scored a mean (SD) of 82.5% (12.8) with one participant being excluded from the study as they proved to be an extreme outlier on this measure scoring only 43%. One further child was unable to fully consume the intervention drink and was also excluded from the analysis. Therefore, a total of 14 children were included in the analysis (10 male) with an average age (SD) of 9.17 years (0.6).

Drink preparation and consumption

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All drinks were prepared on-site at the University of Reading no more than 30 minutes before consumption. The flavonoid-rich blueberry drink contained 143mg anthocyanins and was prepared by mixing 200g of fresh 'Star' variety blueberries with 100ml of semi-skimmed milk and 8 grams of sucrose (to aid palatability). The control drink was matched with the blueberry drink for sugars and vitamin C by adding 0.02g vitamin C powder, 8.22g of sucrose, 9.76g of glucose and 9.94g of fructose to 100ml of semi-skimmed milk.

Participants consumed both drinks with a minimum seven day wash-out period between each separate drink. Order of drink intervention was counterbalanced with children randomly allocated to either the control or blueberry drink before the first test session. Drinks were consumed either at the child's school or their home address using a covered opaque cup and straw in order that the children remained blind to condition.

Cognitive Measures

Given the known peaks in CBF, vasodilation and metabolite availability participants attended the laboratory two hours after they had consumed the test beverages. The total duration of the task battery was 1 hour.

1) ***Go-NoGo*** Participants pressed the space bar each time a target 'mole' (Go) slide was displayed but avoided pressing the space bar when an infrequent 'aubergine' non- target (NoGo) slide was displayed (stimuli courtesy of Sarah Getz and the Sackler Institute for Developmental Psychobiology). Data were analysed using within-subjects T-tests with drink as the independent variable and false alarms, correct go trial, and Response Time (RT) as the dependent variables.
2) ***Rey's Auditory-Verbal Learning Test (RAVLT)*** Participants were played the same pre-recorded list of 15 words (list A) followed by an immediate free recall on five consecutive occasions (recalls 1-5). An interference list (list B) of 15 words was then played to the participants followed by an immediate free recall (interference list recall). Participants

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then performed both a short (2 mins) and long (25 mins, occurring after the tasks described below had been completed) delayed free recall of list A (recalls 6 & 7). Finally, at the end of the test battery, participants performed a word recognition task consisting of a printed list of 50 words containing all the words from lists A and B plus an additional 20 words and asked to circle only the words from list A (word recognition). Two equivalent versions of the RAVLT were used as specified in Lezak, Howieson & Loring [20]. The RAVLT was analysed using within-subjects T-tests for the total acquisition, amount learned, proactive and retroactive interference, and word recognition measures as specified by Lezak et al. A 2(drink) x 5(recall) within-subjects ANOVA was performed to investigate performance over immediate recalls 1-5, whilst a 2(drink) X 2(recall) within-subjects ANOVA was performed to investigate the delayed recalls 6 and 7.

3) ***Word-Colour Stroop.*** The words "Blue", "Red", "Green", and "Yellow" were displayed separately on the screen with each word being displayed in either a congruent or incongruent ink colour. Participants were instructed to press the button on the keyboard which corresponded to the ink colour of the word as quickly as possible. Data were analysed using two separate 2(drink) X 2(congruence) within-subjects ANOVAs with accuracy and RT as dependent variables.

4) ***Visuo-Spatial n-back Task.*** Participants were shown an array of eight "mole holes" displayed in a circle. They pressed a green-coloured key every time a "mole" made a two-back appearance, that is, it appeared in the same "hole" as it had two trials previously. If the "mole" appeared in a different hole from the one it had appeared in two-back then participants pressed a red-coloured key. Data were analysed using two separate 2(drink) X 2(target type) within-subjects ANOVAs with accuracy and RT as dependent variables.

5) ***Object Location Task.*** In this pen and paper task, participants were shown an array of 27 different objects for 1 minute. They were then shown a new array with 20 additional items and given 1 minute to cross through any new items. Participants were then shown an array

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containing only the original 27 items, 16 of which had moved position. They were given 1 minute to circle those that remained in the same place and cross through those which had moved. The original task [21] and a new equivalent version developed for this study, were used. The object and location memory scores were analysed using within-subjects T-tests.

Results

No significant treatment related main effects or interactions were found for the Go-NoGo, Stroop, N-back and Object Location for either reaction time or accuracy responses. However, the RAVLT was shown to be particularly sensitive to flavonoid-induced changes in this sample of children.

As can be seen from Figure 1, regardless of drink type and as would be expected, during the RAVLT word recall improved significantly over the first five successive repetitions of the word list [$F(1,13)=79.14$, $p<.001$]. However, although there was some indication of better performance following blueberry intervention, no significant difference was found between the two drinks for the first five recalls [$F(1,13)=1.01$, $p=.315$], amount of words learned (recall 5 minus recall 1)[$t(13)=-.76$, $p=.46$], final acquisition (recall 5) [$t(13)=1.59$, $p=.136$] or word recognition measures [$t(13)=1.01$, $p=.292$].

Importantly, when considering the delayed memory measures, there was better performance in the blueberry condition than under vehicle. After the short 2-minute delay participants recalled a mean of 10.2 words following the blueberry intervention in comparison to only 8.8 for vehicle; whilst following the 25-minute delay, participants recalled a mean of 9.5 words in comparison to only 8.0 for vehicle. A 2 (intervention) x 2(recall) ANOVA revealed a significant main effect of drink for these recalls [$F(1,13)=5.31$, $p=.038$]. Subsequent post-hoc analysis demonstrated that there was no significant difference between the drinks at the 2-minute delay [$t(13=.12$, $p=.116$], but a trend was found for the direct comparison between drinks at the 25-minute delay recall [$t(13)=1.97$, $p=.07$],

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indicating a positive benefit from blueberry intervention on delayed memory recall**.**

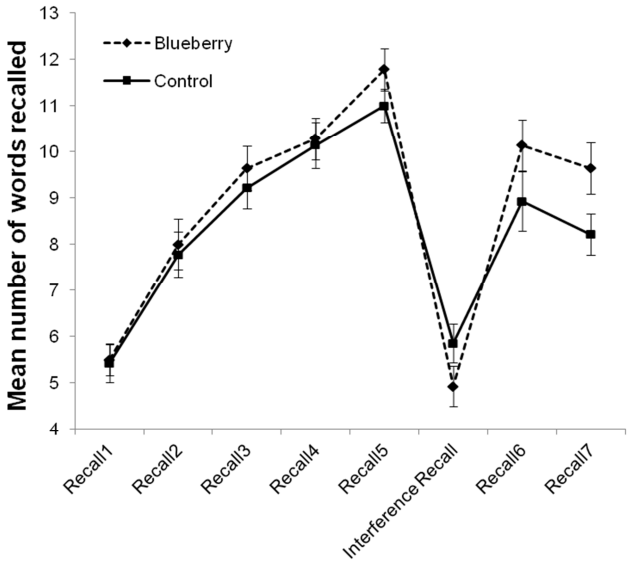

Figure 1. Mean number of words recalled (± standard error of the mean) by recall point and intervention type indicating better list A recall performance following anthocyanin intervention. (Single Column)

As can be seen from figure 2A, however, participants were less affected by proactive interference (PI) following the control drink where there was a decrease in PI (mean=-.42, SD=1.74), contrasting with the blueberry drink where there was an increase in PI (mean=.57, SD=2.06). This difference proved to be significant [t(13)=2.25, *p*=.043]. Looking at figure 1, one can see that this PI effect is being primarily driven by lower performance on interference list recall following the blueberry intervention. However where interference list recall was compared directly, the difference was found to be non-significant [t(13)=-1.79, *p*=.097] indicating that the PI effect found here was primarily an artifact of comparing PI calculations between sessions rather than a true indication of interference. In contrast, there was no significant effect for retroactive interference (RI; figure 2B) [t(13)=-.74, *p*=.474] .

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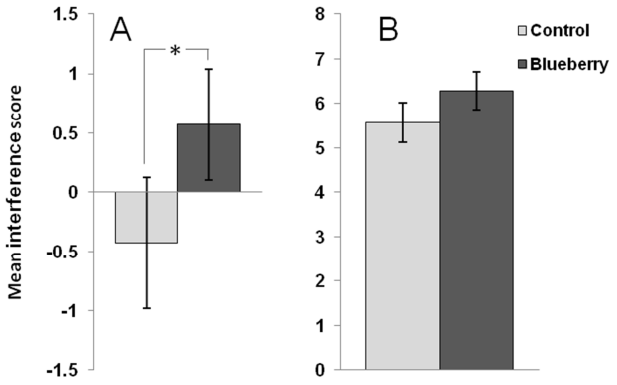

Figure 2. (A) Proactive and (B) retroactive interference levels (± standard error of the mean) following anthocyanin and control interventions (*p<.05). (Single Column)

Discussion

The aim of this study was to investigate the cognitive benefits of an acute flavonoid intervention in a sample of 8-10 year old children. Contrary to previous adult research, we failed to find significant effects on response inhibition, response interference and visual memory tasks [13,14]. We did, however, find a significant improvement in delayed auditory recall performance alongside a negative PI effect following the flavonoid intervention. This gives a preliminary indication that anthocyanin intervention within this particular 8-10 year old age group is sensitive to auditory recall memory measures.

Although a number of mechanisms of action have been proposed for flavonoids, two of the most influential ways of explaining the ways in which flavonoids affect cognitive function are by facilitating an increase in CBF following acute intervention [15] or by facilitating an up-regulation of Brain Derived Neurotrophic Factor (BDNF) [6]. Given that levels of attention are known to be positively related to children's performance on the RAVLT [22], it is possible that a CBF-facilitated increase in oxygen is responsible for improved attentional ability at the point of encoding list A material during the RAVLT. Alternatively, the anthocyanin intervention may have facilitated

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up-regulation of BDNF levels aiding stronger encoding of the words contained in list A of the RAVLT. This would, in turn, have facilitated the improved delayed recall effects we see in these results. Regardless of mechanism, these delayed memory effects are encouraging in relation to the effects of flavonoids on improving retention of verbally delivered material within a learning environment such as a school class.

Furthermore, it could be argued that the positive effects on recall and possible underlying mechanisms may have contributed, in part, to the seemingly negative PI effect. PI is defined as the negative effect of previously encoded material on the encoding of new material and here, it is possible that, following blueberry intervention, the more strongly encoded list A interfered with subsequent encoding of the list B material. Additional research is required to further test this finding. However, it should be noted that a direct comparison of interference list performance on its own proved to be non-significant indicating that the PI effect may not be as strong as the RAVLT PI calculation would imply.

In his review on polyphenol intervention, Lamport [4] notes that, of the cognitive areas investigated, declarative memory would seem to be the most sensitive to polyphenol intervention, which is, in part, what was found in this study. However, no significant effects were found for any other task from our relatively large cognitive battery where one might also have expected to see improvements in relation to previous adult studies [13,14]. The attention-related response interference/inhibition tasks used on this occasion, however, were relatively simple. This may have been pertinent given that other flavonoid related studies have shown performance following intervention is to be particularly sensitive to task (e.g., [23]). Indeed, where the cognitive task is simple, it has been found that, following flavonoid intervention, participants demonstrate increased brain activation during the task, however, this may not necessarily translate into a differentially better performance [15]. Furthermore, being a pilot study, the small sample size in this study may also have precluded finding significant effects in the less sensitive tasks. It is therefore recommended that future studies should

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bear task sensitivity in mind along with a larger sample in order to reliably replicate the effects found here.

It should be noted that, on this occasion, only one dose and time-point were investigated. This is particularly relevant given that other flavonoid related studies have shown that attention related performance can be influenced both positively and negatively in relation to dose [14]. Further multi dose/time point studies are therefore now required to fully understand the impact of flavonoid intervention on cognitive performance.

Conclusion

This pilot study reporting the effects of blueberry anthocyanins on the cognitive behaviour of primary school aged children indicated that a 143mg blueberry anthocyanin dose benefits delayed recall but may negatively influence pro-active interference in 8-10 year olds. There was, however, little evidence of an effect for more direct measures of attention and visuo-spatial working memory and further research is recommended in order to test the acute cognitive effects of blueberry anthocyanins at different dose/duration within this age group.

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Highlights

- Cognitive performance of 8-10 year olds is tested following acute flavonoid intervention
- Delayed memory recall improves in comparison to control
- Proactive Interference increases in comparison to control
- No effect found for executive function tasks
- Cognitive benefits found following acute flavonoid supplementation

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