



Pilot study

Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10 y old children



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ABSTRACT

Objective: Recent evidence from animals and adult humans has demonstrated potential benefits to cognition from flavonoid supplementation. The aim of this study was to investigate whether these cognitive benefits extended to a sample of school-aged children.

Method: Using a crossover design, with a washout of at least 7 d between drinks, 14 children ages 8 to 10 y consumed either a flavonoid-rich blueberry drink or a matched vehicle. Two h after consumption, the children 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.

Results: In comparison to the 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 memorized 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 visuospatial memory.

Conclusions: Although 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. 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 [1–4]. For example, chronic supplementation with both

young and aging rodents has shown improved visuospatial memory in T, radial, and Morris water maze tasks [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 [4] reporting that positive visuospatial memory effects have been found in a number of adult studies [8,9]. The review [4] also noted that immediate verbal memory would seem to be particularly sensitive to chronic flavonoid or polyphenol berry fruit juice intervention in adults [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 also have been found after acute flavonoid intervention, 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),

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protecting against neuronal stress, and positively mediating signaling pathways in the brain [2,6,15,16]. Here, research indicates that peak increases in CBF occur 2 h after acute cocoa flavonol intervention [15], whereas specific increases in endothelium-dependent vasodilation and availability of anthocyanin metabolites can be seen at 1 to 2 and 6 h after acute blueberry anthocyanin intervention [17].

Although 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 and 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 children ages 8 to 10 y.

Material and methods

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

Participants

An opportunity sample of 16 children in year 4 (age range 8.08–9.83 y) were recruited from two primary schools local to Reading University. Participants were screened in advance for fruit allergies, dyslexia, and attention-deficit hyperactivity disorder. As a further control, all participants performed a computerized version of the Raven's Progressive Matrices to provide a measure of fluid intelligence. Here, participants scored a mean of 82.5% (SD 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, 14 children were included in the analysis (10 boys) with an average age of 9.17 y (SD 0.6).

Drink preparation and consumption

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

Participants consumed both drinks with a minimum 7-d washout 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 at the home address using a covered opaque cup and straw so that the children remained blind to condition.

Cognitive measures

Given the known peaks in CBF, vasodilation, and metabolite availability, participants attended the laboratory 2 h after consuming the test beverages. The total duration of the task battery was 1 h.

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 analyzed using within-subject's *t* tests with drink as the independent variable and false alarms, correct go trial, and response time (RT) as the dependent variables.

Rey's auditory-verbal learning test

Participants were played the same prerecorded 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 then performed both a short (2 min) and long (25 min, occurring after the tasks described below had been completed) delayed free recall of list A (recalls 6 and 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. They were asked to circle only the words from list A (word recognition). Two equivalent versions of the Rey's Auditory-Verbal Learning Test (RAVLT) were used as previously specified [20]. The RAVLT was analyzed using within-subject's *t* tests for the total acquisition, amount learned, proactive and retroactive interference, and word-recognition measures as previously specified. A 2(drink) × 5(recall) within-subject's analysis of variance (ANOVA) was performed to investigate performance over immediate recalls 1 to 5, whereas a 2(drink) × 2(recall) within-subject's ANOVA was performed to investigate the delayed recalls 6 and 7.

Word-colour stroop

The words "blue," "red," "green," and "yellow" were displayed separately on the screen with each word being displayed in either congruent or incongruent ink colors. Participants were instructed to press the button on the keyboard that corresponded to the ink color of the word as quickly as possible. Data were analyzed using two separate 2(drink) × 2(congruence) within-subject's ANOVAs with accuracy and RT as dependent variables.

Visuospatial n-back task

Participants were shown an array of eight "mole holes" displayed in a circle. They pressed a green-colored 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-colored key. Data were analyzed using two separate 2(drink) × 2(target type) within-subject's ANOVAs with accuracy and RT as dependent variables.

Object location task

In this pen-and-paper task, participants were shown an array of 27 different objects for 1 min. They were then shown a new array with 20 additional items and given 1 min to cross through any new items. Participants were then shown an array containing only the original 27 items, 16 of which had moved position. They were given 1 min to circle those that remained in the same place and cross through those that had moved. The original task [21] and a new equivalent version developed for this study were used. The object and location memory scores were analyzed using within-subject's *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 RT 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 in 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 ($F_{1,13} = 79.14, P < 0.001$). 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 ($F_{1,13} = 1.01, P = 0.315$), amount of words learned (recall 5–recall 1) ($t_{13} = -0.76, P = 0.46$), final acquisition (recall 5) ($t_{13} = 1.59, P = 0.136$), or word recognition measures ($t_{13} = 1.01, P = 0.292$).

Importantly, when considering the delayed memory measures, there was better performance in the blueberry condition than under the vehicle. After the short 2-min delay, participants recalled a mean of 10.2 words following the blueberry intervention compared with only 8.8 for the vehicle; whereas following the 25-min delay, participants recalled a mean of 9.5 words compared with only 8 for the vehicle. A 2 (intervention) × 2 (recall) ANOVA revealed a significant main effect of drink for these recalls

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