Systematic review of the effects of blueberry on cognitive performance as we age

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Abstract

The effect of flavonoid-rich food, such as blueberries, on cognitive function has been subject to a growing amount of research interest in recent years. Epidemiological, prospective, pre-clinical and clinical trials have revealed positive cognitive benefits from flavonoid interventions, particularly in relation to the amelioration of cognitive decline in older adults. This review will specifically consider the existing clinical research from both acute and chronic blueberry interventions on cognition in human subjects. The results of 11 studies are reported with four studies considering blueberry intervention with 7 – 10 yr old children, four considering aging 60+ yr old adults, and three considering adults suffering from mild cognitive impairment (MCI). Findings from these studies indicate that cognitive benefits may be found for delayed memory and executive function in children and for delayed memory, executive function, and psychomotor function in older healthy and MCI adults. There is less evidence to suggest positive benefits of blueberry intervention on working memory. Recommendations for future research including dose used, cognitive tasks, and age groups considered, are proposed.

Keywords: Flavonoids, blueberry, cognition, life-course, anthocyanins

Introduction

Blueberries have been the subject of a number of health-related research studies in recent years with supplementation showing reduced risks for metabolic syndrome, cancer, cardiovascular disease and also cognitive decline (1). Mechanistically, initial research (2, 3) focused on the anti-oxidant properties of flavonoids and their ability to combat oxidative stress (OS). However, recent studies have suggested a number of other mechanisms by which flavonoid-rich interventions may promote cognitive health (for a review see (4)). Indeed, recent mechanistic research has shown that the health benefits of blueberries may be ascribed to their particularly high flavonoid content. As can be seen from table 1, blueberries are particularly high in anthocyanins along with lower amounts of

flavanols and flavonols, all of which are flavonoid subclasses (5). They also contain small quantities of phenolic acids, in particular chlorogenic acid (5). Although there is evidence of higher anthocyanin content in other berries such as chokeberries, the ease of blueberry availability and also their relatively better palatability make them ideal candidates for flavonoid-rich intervention studies. Previous studies have documented that lowbush (typically called 'wild') and highbush blueberry varieties differ in taste, size and flavonoid content. Lowbush blueberries tend to be smaller, have a more intense flavour, and are usually found growing wild in colder and harsher climates whilst high-bush blueberries tend to be bigger and grow in abundance. For this reason, highbush blueberries tend to be the first choice for commercial cultivation, however high performance liquid chromatography-mass spectrometry (HPLC-MS) has revealed that the lowbush variety contains approximately three times the amount of phenolic compounds found in highbush varieties (6, 7). Many different factors could account for these differences; such as cultivar, cultivation practise, environmental growing conditions, processing and storage (8-11).

Table 1 here

In recent years, blueberries have gained significant attention for their ability to promote better cognitive performance and also contribute to a delay in cognitive decline as we age. Epidemiological studies suggest that intake of flavonoid-rich foods, such as blueberries, ameliorates cognitive decline during ageing. For example, Letenneur et al. (12) investigated the effects of flavonoid consumption in a group of 1640 older adults aged 65+ over a 10-year period finding better cognitive performance in participants who consumed greater amounts of dietary flavonoids. Similarly, the Nurses' health study (13) monitored cognition and dietary intake over 20 years in a cohort of 16,010 women, aged 70 or above, finding increased consumption of blueberries was related to slower cognitive decline. Both studies support the notion that flavonoids not only have a beneficial effect on cognition during aging, but also may offer neuroprotective properties.

In addition to this epidemiological data, various pre-clinical animal studies have been conducted to investigate the effects of flavonoids derived from berries on cognition. In one of the first studies of its kind, Joseph et al. (14) found blueberry, strawberry, or spinach supplementation for 8 weeks in mice with neurodegeneration resulted in a reversal of neuronal ageing which they attributed to a reduction oxidative stress damage. Whilst this was one of the first studies to document a potential mechanism of action for positive cognitive effects following blueberry supplementation, as outlined above, other possible mechanisms have also been described in recent years including the flavonoid-induced upregulation of neuronal signalling proteins. For example, work from our lab (15) found that 18-month old rats supplemented for 12 weeks with blueberry showed elevated hippocampal levels of cAMP-response element-binding protein (CREB), extracellular signal-related kinase ERK1/2, and brain-derived neurotrophic factor (BDNF) in comparison to age-matched controls. Importantly, the alteration in these signalling proteins was accompanied by better performance on a spatial working memory cross maze task. In a follow-up study, we demonstrated that the beneficial effects of blueberry supplementation could also be found on spatial working-memory tasks in younger rats

aged 2 months, again accompanied by increased hippocampal levels of BDNF, CREB and ERK1/2 activation (16). BDNF seems to be a particularly important potential mechanism of action for blueberry supplementation. In humans BDNF levels are known to decrease across the day (17, 18) however, research by Dodd (19) has found that, in both younger (18 – 25 yrs) and older (62 – 73 yrs) adults, plasma levels of BDNF are maintained following blueberry intervention compared to placebo treatments where levels decrease. Furthermore, BDNF is thought to play a critical role in the delay of ageing by improving hippocampal plasticity as well as increasing neurogenesis and long-term memory (20). Therefore, these pre-clinical studies (17, 18) and the findings by Dodd (19) suggest a possible mechanism of action whereby blueberry intervention contributes to the maintenance of BDNF availability which may be critical for cognitive function. Pre-clinical studies have also provided evidence that blueberry flavonoids may exert a positive effect on neuroinflammation. For example, Shukitt-Hale et al. (21) found that, following infusion of kainic acid (KA) to the hippocampus, 4-month-old rats supplemented with blueberry performed better on a Morris water maze task and showed a reduced inflammatory response to the KA insult. Finally, Casadesus et al. (22) found that, when compared to placebo group, there was increased hippocampal neurogenesis alongside improved spatial memory performance in aged rats following 8 weeks blueberry supplementation.

Taken as whole there are a number of possible mechanisms underpinning the beneficial cognitive effects of blueberry intervention, these include antioxidant and anti-inflammatory actions, up-regulation of neuronal signalling proteins, and stimulation of neurogenesis. Further details of pre-clinical studies which have considered the potential mechanisms of action underpinning blueberry intervention can be found in reviews by Miller et al. (23), and Pribis and Shukitt-Hale (24). However, evidence would suggest that, irrespective of mechanism of action, the positive effects of blueberry intervention can be found primarily in the hippocampus, a brain area critical for optimal memory function (15, 22, 25, 26).

From the pre-clinical research described above, there is good evidence from both a mechanistic and behavioural level to suggest that blueberry intervention should facilitate improved cognitive performance in clinical trials. Therefore, this review will assess the evidence from both acute and chronic intervention studies for the beneficial effects of blueberry on human cognitive functioning across the lifespan. The domains of cognitive function found to be sensitive to blueberry interventions will be identified and, based on the reported research, cognitive areas which have yet to be considered will be highlighted. Tentative recommendations regarding future research directions will also be made.

Method

An electronic search of PubMed, Google Scholar and Web of Science was conducted using the search terms Blueberr* and/or Berr*, Anthocyan* and/or Flavonoid*, Cognit* and/or Polyphenol* and/or Memory and Executive function. The studies selected for inclusion were all subject to peer- and/or editorial-review, and for this reason, conference abstracts have been omitted. Papers published in the English language, with no restriction on publication date, were selected, and subsequently the bibliography of each paper was scanned to reveal further possible papers. The following inclusion and exclusion criteria were implemented:

- *Inclusion:* Human studies, participants of all ages, healthy participants/participants with MCI, studies measuring the effect of blueberries on cognitive function, cognition measured using appropriate cognitive tasks, all forms of blueberry treatment including juice, fresh, powder, extract, and smoothie.
- *Exclusion criteria*: Epidemiological studies, participants with neurodegenerative diseases such as Alzheimer's, animal studies, studies using more than just blueberries e.g mixed berry drink.

Results

In total, 11 studies considering the cognitive effects of blueberry intervention were found (summarised in Tables 2-4). The primary cognitive domains considered were episodic memory (EM), working memory (WM), executive function (EF), and psychomotor function (PF), although a wide range of different tasks were used to test these cognitive domains (Note: detailed descriptions of the tasks used can be found in Supplementary information). No studies were found considering the effect of blueberry interventions on young or middle aged adults with the research to date focusing exclusively on children aged 7 – 10, healthy older adults aged 60+, or older adults exhibiting symptoms of Mild Cognitive Impairment (MCI).

The effects of blueberry polyphenols in children

Four studies have investigated the effect of blueberry interventions on the cognitive function of children aged between 7 – 10 years of age (Table 2). All four of the studies considered the acute effects of blueberry intervention and, to date, no published studies have considered chronic repeated administration designs.

The first study considering the acute effects of blueberry intervention on children was performed by Whyte and Williams (27). In this crossover trial, a blueberry-based drink was administered to a group of 14 children aged 8-10 years old. The drink consisted of 200g fresh highbush blueberries, blended with milk, giving a reported total anthocyanin concentration of 143 mg. This was a randomised crossover study design, with a 7-day washout period between the two study days. Baseline testing was not employed in this study but instead, on each study day, the cognition of each child was tested at 2 hours post consumption of either the blueberry or placebo drink. The cognitive tasks used in this study included the Go-NoGo, Stroop, Auditory Verbal Learning Task (AVLT), Object Location Task, and Visual N-back.

The study yielded no significant effects for accuracy and reaction times (RT) of blueberry intervention on any of the outcome measures in the Go-NoGo, Stroop, N-back or the object location task. However, ANOVA analysis of the AVLT revealed a significant benefit of blueberry, in comparison to placebo, for the main effect across short and long delayed word recall. Further post-hoc analysis revealed a positive trend for better recall following blueberry after a 25-minute delay indicating a sensitivity to delayed recall following blueberry intervention in children. In terms of proactive interference (PI) there was evidence that performance was less affected following the placebo drink compared to blueberry. However, when the interference recall list performance was directly compared no significant difference was found leading the authors to conclude that this effect was more likely an artefact of the PI calculation when applied across two separate test sessions.

In a follow-up RCT within subjects study, Whyte et al. (28) considered the effects of two wild blueberry interventions of 15 and 30g (anthocyanin content of 127mg and 253mg respectively), or matched placebo on cognitive performance in 7 – 10 yr old children. On each study day cognitive tests were performed at baseline, then 1.5, 3, and 6 hrs following intervention. The four cognitive tasks used were the AVLT (as above), Modified flanker Task (MFT), Go-NoGo (as above), and Picture Matching Task (PMT). Analysis revealed dose response effects for both memory and EF measures. Memory effects included a significant interaction for the AVLT measure of final acquisition of the word list, with post-hoc analysis revealing significantly better 30-g WBB performance at 1.5 hrs in comparison to placebo. Additionally, for delayed word recognition, although there was a decrease in performance across the test day for all three treatments, there was a significant main effect of dose with the placebo performing least well overall. Furthermore, post-hoc analysis revealed the difference between placebo vs 15-g WBB and between placebo vs 30-g WBB to be greatest at the 6hr time point. Executive function effects included a significant effect 3hrs after the intervention on the incongruent trials of the flanker task where, compared to baseline performance, 30-g WBB performance improved, placebo performance deteriorated, and there was no change for the 15-g

treatment. Analysis of the data also revealed significant linear trends for Final Acquisition, Word Recognition, Incongruent MANT trials, and Picture Name Matching Trials, with the placebo performing least well, followed by the 15-g WBB and then the 30-g WBB performing best in all cases. Given this evidence of a dose response effect, a non-parametric Page's test was conducted on combined scores from all tasks and session revealing a monotonic increase in cognitive performance in relation to WBB dose.

A more recent study by Whyte et al. (29) focused on the cognitive effects of a 30g WBB treatment (containing 253 mg anthocyanins) coinciding with the 3-hr point at which positive cognitive EF effects were found in the previous Whyte et al. (28) paper. The aims of the study were to explore the effects of varying demand on cognitive performance following a flavonoid rich WBB intervention. This study employed the Modified Attention Network Task (MANT), an executive function task that can be manipulated to vary cognitive demand/load across a number of different factors such as congruency, visual load, distractor noise, target duration, and target cueing. The results revealed that following WBB intervention, there was a significant global effect whereby children responded to the stimuli significantly faster, compared to placebo. Furthermore, it was found that WBB cognitive performance was better in comparison to placebo at the slower 500ms presentation rate during the more cognitively demanding, high visual load incongruent trails, supporting the study's hypothesis. However, cues alerting the appearance of the target also facilitated significantly better WBB performance in comparison to placebo. In contrast to the earlier findings of Whyte et al. (28), no effects on accuracy were found for this EF task.

Barfoot et al (30) looked at the acute effects (2 hrs) of blueberries on executive function using the MANT on a group of 54 children aged 7-10. In this study they employed a single-blind, parallel-groups design. As well as executive function, they tested the effect on verbal memory using AVLT and reading efficiency using Test of Word Reading Efficiency-2 (TOWRE-2). Mirroring the findings of Whyte et al. (28) executive function benefits were found with significantly faster reaction times on

the MANT following WBB treatment, although on this occasion, the benefits were found on the trials presented at the faster 120 ms rate with no loss in accuracy. Furthermore, similar to the AVLT final acquisition and delayed recall benefits found by Whyte et al. (28), total acquisition performance was improved following the blueberry intervention, with significant improvements seen on the short delay trials. There were no effects seen for any of the TOWRE-2 parameters.

In summary, four studies have considered the impact of blueberry interventions on cognitive performance in children (however see Khalid et al. (31) for positive effects on a measure of mood). These studies have found evidence that executive function and delayed memory performance are positively affected by blueberry treatment in comparison to placebo. It should be noted that, to date, only a narrow age range, between 7 – 10 yrs has been considered with effects in infants and teenagers yet to be explored.

Table 2 here

The effects of blueberry polyphenols in healthy older adults.

Four studies have been conducted looking at the effects of blueberry on cognition in healthy older adults. This research is in line with a wider and growing body of research considering the delay of cognitive decline in older adults (32-34). Previous findings from pre-clinical experiments looking at the effects of blueberry on the cognition of aged animals showed a positive change in working memory performance as well as improved mobility in aged rats (14, 25). Building on these findings, Miller et al. (35) considered the effect of a 24g freeze dried blueberry (19.2 mg/g anthocyanins, equivalent to 460 mg anthocyanins daily) intervention on the cognitive performance and mobility of adults aged 60-75 years old for a total of three months (n=37). The study consisted of a parallel design, and measurements were taken at day 1, day 45, and day 90. Note, for the purpose this review, only cognitive outcomes will be discussed. The cognitive tasks included in this study were the Task-switching test (TST), Trail making test (TMT), California Verbal Learning Test (CVLT), Digit span (DS) task, a virtual version of the Morris Water Maze (vMWM), and Attention Network Task (ANT).

Results for TST generated no significant differences between treatments for reaction times. In terms of accuracy, a reduction in switch cost was indicated whereby there was a significant visit by treatment interaction with participants in the blueberry condition showing a reduction in switch trial errors over the test visits in comparison to placebo. In the CVLT, participants improved significantly on the number of words correctly recalled regardless of treatment. However, there was a significant visit by treatment interaction with participants in the blueberry group making fewer repetition errors on day 90 than they did on day 0. Participants in the placebo group showed the opposite pattern making more repetition errors at day 90. There were no significant treatment related effects of blueberry on any of the other stated outcomes measures.

Schrager, Hilton, Gould and Kelly (36) considered the positive effects of blueberry intervention on motor and psychomotor function along with tests of executive function. Twenty unblinded participants were randomly allocated to either a daily regimen of 2 cups of blueberries (n = 13) or a carrot juice placebo (n = 7) for 6 weeks. The polyphenol and anthocyanin content of the blueberry intervention were not stated. The cognitive tasks used were the Simple Reaction Time, TMT B, and Dual-Task adaptive gait test (DTAG). Further measures of grip strength, gait speed and adaptive gait were also recorded. Analysis of the results revealed a significant improvement in executive function following blueberry treatment with participants performing less step errors during the DTAG in comparison to the placebo condition. There were no other treatment related effects for the cognitive tasks in this study, however the participants also showed improved motor function related to increased gait speed following blueberry intervention. It should be noted that there are possible issues with the control drink used in this study. Although not a rich source of flavonoids, carrots are abundant in carotenoids and other polyphenolic compounds. Research has shown that the carotenoids, lutein and zeaxanthin, are associated with improved cognitive function (37, 38) whilst long-term supplementation with beta-carotene influences cognition (39). Besides these issues with components in the placebo treatment that are known to influence cognition themselves,

participants were also unblinded to the treatment they received. Given these concerns over the design, some caution should be employed in consideration of the findings here.

Bowtell et al. (40) looked at the effects of blueberry supplementation on cerebral blood flow, with cognition as a secondary outcome. The study adopted a parallel, double blind design, testing the effects in 26 healthy adults, with an average age of 68 yrs, after 12 weeks of supplementation. The cognitive battery consisted of 6 tasks: 1) Detection task, 2) Groton maze timed chase test, 3) Groton maze learning test, 4) Identification task, 5) International shopping list task, and 6) 1-back and 2-back task. To measure brain activation, functional magnetic resonance imaging (fMRI) was performed whilst the participants conducted a numerical Stroop task. Arterial spin labelling (ASL) measures of brain perfusion were also gathered whilst the participant was in a rested state. Analysis revealed no significant treatment-related effects on the Detection Task, Groton Maze tests, Identification task, shopping list task, and Stroop test, however, there was a trend towards better performance in the 1-back test and trends for improved reaction time and accuracy on the 2-back test. Although there were no significant treatment-related behavioural effects whilst performing the response interference Stroop task, fMRI analysis revealed significant increases in activation of a number of task related areas (Brodman areas 4, 6, 10, 21, 40, 44, 45, precuneus, anterior cingulate, insula, and thalamus) in comparison to baseline. No such effects were found for the placebo. Furthermore, the resting state ASL analysis revealed increased grey matter perfusion in the parietal and occipital lobes following blueberry whereas, again, no effect was found for the placebo.

A recent study by Whyte et al. (41) compared three blueberry treatments stabilised with L-cysteine and L-Glutathione with placebo on measures of cognitive function, cardiovascular function and mood. A total of 112 healthy, older participants completed the 6-month long study consuming two capsules of their allocated treatment per day, with testing occurring at baseline, 3 months and 6 months. The participants were randomised into 4 treatment groups consisting of placebo, 500 mg wild blueberry powder (WBB), 1000 mg WBB, and 111 mg wild blueberry extract (WBE) containing 0,

1.35, 2.7 and 7 mg anthocyanin content and 0, 35, 70 and 50 mg polyphenols, respectively. Although the anthocyanin content is lower than what would be present from a single serving of fresh blueberries, all treatments had L-cysteine and L-Glutathione added to them in order to facilitate the stabilisation of their anthocyanin content and, in turn, allow a higher rate of absorption than might be possible via general habitual intake or at doses used in previous studies. In terms of cognitive testing, the primary outcome measure was episodic memory, via three different tasks including the RAVLT, object recognition task, and Corsi Block task. The secondary cognitive outcome measures tested executive function, attention and working memory. Tasks included the Serial 3's and 7's, Sternberg memory scanning task, MANT, and Stroop task.

Linear mixed model analysis revealed that for the word recognition measure of the AVLT, there was a significant treatment by time interaction with post hoc analysis finding improvement after supplementation with WBE compared to the placebo after 3 months, but not after 6 months. There were no significant differences for the other blueberry treatments for this measure. A similar pattern of results was found for the total number of Corsi Block sequences correctly recalled where there was a significant treatment by time interaction with post hoc analysis finding a trend for improvement following supplementation with WBE compared to the placebo after 3 months, but not after 6 months. Again, there were no significant differences for the other blueberry treatments for this measure. For the working memory and executive function tasks, there were no significant effects for any of the blueberry treatment compared to placebo at any of the time points. In terms of the markers of cardiovascular health, a main effect of intervention was found with post-hoc analysis revealing significantly lower WBE systolic blood pressure over the 6-month intervention, however, no such effect was found for the other blueberry treatments. It is interesting, and somewhat unexpected, that no significant differences in cognitive performance were seen after 6 months and the authors posit that this may reflect an element of practice whereby participants improved their strategy to perform these tasks over time with repeated exposure thus reducing task sensitivity to the intervention.

In summary, four studies have investigated the effects of pure blueberry intervention in older adults. Results from these studies have been mixed. Of the three studies which considered episodic memory, only two (35, 41) found significant effects in the different subdomains of delayed recognition and repetition errors. All studies considered executive function, though positive behavioural benefits were only found in two. It could, however, be argued that the effects were found on the more cognitively demanding switch trials of the switching task (35) and the Dual Task Adaptive Gait Test (36). Furthermore, the elevated brain activation, which was found in the absence of significant behavioural effects during performance of the less cognitively demanding Stroop task (40), gives further indication that in order to establish blueberry related executive function benefits within an older age group, the level of task demand should be carefully considered.

Table 3 here

Adults with mild cognitive impairment

Similarly to the other age groupings assessed in this review, there are few studies considering the effects of blueberry supplementation in adults with mild cognitive impairment. To date, only 3 studies fulfilled our inclusion and exclusion criteria.

Krikorian et al. (33) investigated for the first time the effects of blueberry on cognition on older adults with MCI. The sample size was a total of 9 participants with a mean age ±72 along with the data from a placebo group of 7 participants gathered from a previous Concord grape juice study (42). The study employed a randomised, double blind, placebo-controlled trial testing the effects of blueberry for 12 weeks. Dosage of blueberry treatment was calculated according to body weight, more specifically, participants weighing 54-64kg received 444 mL/day and participants in 65-76 kg received 532 mL/day, participants weighing 77-91 kg received 621 mL/day, the placebo was a grape-flavoured drink that contained no polyphenols. Cognitive measurements from both treatment groups were taken at baseline and 12 weeks and the participants performed a battery consisting of two tasks testing verbal learning and memory which are known to be processed by the hippocampal

region. The tasks included Verbal Paired Associate Learning Test (V-PAL) and the California Verbal Learning Test (CVLT).

Analysis of the V-PAL cumulative learning scores showed the cumulative score significantly improved at 12 weeks compared to baseline, as did delayed recall performance during the CVLT. However, no mention is made regarding these comparisons for the placebo either in this paper or the companion study from which the placebo group data was drawn. This raises the possibility that the findings were primarily practice effects. Acknowledging this possibility Krikorian et al. performed a further comparison with the placebo group on the 12-week time point data which found significantly better V-PAL performance for those receiving the blueberry intervention, however no such effect was found for the CVLT data. This would indicate that whilst the V-PAL effects would seem to be robust, the effects reported for the CVLT should be considered with caution. Furthermore, although the results yielded significant effects, this was a small study (n=16 which includes data from a placebo group from a different study).

Boespflug et al. (32) measured working memory performance in a group of MCI participants aged 68-92 (n=16). Additionally, brain activation was assessed using fMRI whilst the participants conducted a cognitive task. The study employed a randomized, double-blind, placebo-controlled parallel study. The cognitive task used was the sequential letter n-back, assessing working memory, as in Bowtell et al. (40). In this study, there were three different conditions; 0-back, 1-back, 2-back with the outcome measures being accuracy and reaction time. The treatment was administered as a drink consisting of water and blueberry powder giving a daily dosage of 269 mg of anthocyanins, equivalent to roughly 220g of fresh blueberries. This was administered daily for a total of 4 months, with measurements taken at pre-intervention (baseline) and post-intervention (week 16).

Analysis of working memory performance revealed that for all the 0-back and 2-back conditions there were no significant improvements in reaction time at any of the timepoints. For the 1-back condition, there was a trend towards significance ($p = .08$) for accuracy in the blueberry group

compared placebo group performance at 16 weeks. However, though a significant difference was found at baseline, with placebo performing significantly faster than blueberry in the 1-back condition, no reaction time differences were found between treatments post-intervention. In terms of fMRI results, there were observed changes in the blueberry treated group, with increased activation in the left pre-central gyrus, left middle frontal gyrus, and left inferior parietal lobe during the final visit (16 weeks). More specifically, analysis revealed a significant increase of signalling in the left inferior parietal gyrus and left pre-central gyrus during the 2-back condition for blueberry treated group. There were, however, no significant effects of activation under the 0-back and 1-back conditions. In terms of the placebo group, decreased activation was witnessed close to the left post-central gyrus at the final visit compared to baseline.

A more recent study by McNamara et al. (34) investigated the effects of blueberry supplementation, fish oil, and a combination of the two on the cognition of 94 healthy men and women aged between 62-80 years old who had not been diagnosed with any form of cognitive impairments, but did suffer from self-reported cognitive complaints. The sample population was divided into four groups; blueberry powder + placebo oil; fish oil + placebo powder; blueberry powder + fish oil; and placebo powder + placebo oil. The blueberry treatment was equivalent to 25g dry weight of blueberry a day and provided 269 mg of anthocyanins per serving. The intervention lasted a period of 24 weeks with measurements taking place at week 0 (baseline) and week 24, as well as an additional measurement 24 weeks after the intervention period (week 48). A total of 76 participants completed the whole study successfully and a total of 65 took part in the post-intervention measurements. Cognitive assessments used included Dysexecutive Questionnaire (DEX) to assess executive function, Trail making tests A and B (TMT-A and TMT-B), Controlled Oral Word Production, and Hopkins Verbal Learning Test (HVLT*)* to assess verbal learning and long-term memory. As well as cognition, there were measurements of red blood cell fatty acid composition, anthocyanin levels in urine, metabolic factors, *APOE* genotyping as well as anthropometrics measurements. In the context of this review, only the cognitive outcomes will be discussed.

Results found that for the DEX, the scores for the blueberry treated group decreased significantly, indicating that fewer negative cognitive symptoms experienced in everyday activities at 24 weeks. This benefit was maintained at the 48 week point, 24 weeks following the cessation of treatment. Furthermore, the blueberry treated group displayed improvements in HVLT recognition memory discrimination performance after 24 weeks, however, this effect was not maintained at 48 weeks. There were no significant blueberry-related improvements for any of the other tasks. Comparing this with the other interventions, the DEX scores also decreased significantly for the fish oil-treated group whilst increases on DEX were seen following placebo. As for the other cognitive tasks, no significant improvements were observed for either fish oil, the combined fish oil and blueberry group, or the placebo groups. Overall, the researchers concluded that, in a sample of older adults experiencing self-diagnosed cognitive complaints, the blueberry intervention improved cognitive efficiency for everyday life activities, as well as improving resilience against extraneous disturbances during a recognition memory task.

Of the two studies above which considered episodic memory, both found positive effects following blueberry intervention. Interestingly, the recognition memory performance found in healthy adults by Whyte et al. (28) was also found by McNamara et al.(34) in adults suffering from mild cognitive complaints, indicating the sensitivity of this measure to blueberry intervention in an aging population. There was little evidence of a working memory effect, with the results of Boespflug et al.(32) only trending towards significance, however, in a similar fashion to Bowtell et al. (40) fMRI analysis again showed elevated task related brain activation despite no behavioural effects being found.

Table 4 here

Discussion

Studies investigating the effects of blueberry intervention to date have been limited and have only considered two main age groups, children aged between 7 – 10 years old or older adults aged 60 and

above. This latter group can be further subdivided in to healthy adults and those with MCI. As regards study type, only acute (single administration) interventions have been published with children whilst only chronic (repeated administration) interventions using varying durations of treatment have been published with older adults. Tasks employed have differed between studies with some considering only one cognitive domain and others a wider range. Furthermore, anthocyanin doses employed have ranged from 1.35 mg to 460 mg in chronic studies and between 143 mg and 253 mg in acute studies. Cognitive results from these studies have been mixed, with results not being seen consistently across the different domains considered, though see below for comments on task sensitivity. Furthermore, as can be seen from Tables 2-4, there was a spread of effect sizes with Cohen's d ranging between .175 and 1.94. Making any strong conclusions regarding expected cognitive outcomes in relation to developmental stages and proposing best practice for future research is therefore not possible given the literature available. The following discussion should therefore be considered in this light.

Within the domain of memory, benefits have primarily been found on episodic measures with significant improvements being found for acute child interventions on the AVLT in word acquisition, delayed recall, and word recognition (27, 28, 30). Interestingly the effect of improved episodic memory performance is also seen in chronic older adult interventions with a number of studies finding positive effects on either the CVLT, HVLT, or AVLT measures of episodic memory (). It should be noted that the above studies were the only ones to include measures of episodic memory in the task batteries used and, in all cases, at least one sub-domain was positively affected by intervention. This gives some indication that episodic memory is particularly sensitive to anthocyanin blueberry intervention in both children and older adults. It should be noted however that, in a review of the literature considering acute flavonoid interventions of all classes, Bell et al. ((43)) reported there was little evidence of a positive episodic memory effect in young adults and further blueberry related research is therefore required to clarify whether episodic memory effects might also be found within this age group.

The positive benefits of executive function (EF) are also present in the literature for both age groups with children showing improved performance following blueberry intervention on the more cognitively demanding response interference trials of the modified flanker and modified attention network tasks (28, 29). In older adults the results are more equivocal with only two out of the four studies which measured EF reporting blueberry related effects. It should be noted that where there were significant findings, the effects were found on arguably the more cognitively demanding elements of the tasks with results being found on the critical switch trials of the switching task (35), and the dual task adaptive gait task involving the simultaneous performance of two tasks at once (36). When considered together, the results of both the acute and chronic studies indicate that blueberry intervention may have an effect on EF, however, task sensitivity is critical with the improvements becoming more evident between treatment and placebo as the cognitive demand of the task increases. This highlights the importance of task sensitivity and demonstrated that some tasks used in the studies presented in this review may not have been sufficiently demanding for differences in performance to be observed between treatment groups.

Tests of working memory have revealed no evidence of blueberry related benefits in children (27, 28). For older adults benefits of WM were found in two of the four studies which considered this domain with the effects being seen either on 1 or 2 n back tasks (32, 40) however, it should be noted that these effects were trends and, in both studies, there was no reported statistical correction for the analysis of the multiple n-back versions employed. Taken as a whole, therefore, the literature would suggest there is little benefit to be found for blueberry intervention within this domain.

One explanation for the benefits found in cognitive function could be due to improved memory encoding as a result of elevated levels of BDNF. Pre-clinical studies have shown blueberry's efficacy in increasing the level of brain-derived neurotrophic factor (BDNF) in the hippocampal area of the brain (15, 16). BDNF is a neurotrophin, a protein that plays an important role in cell regeneration, differentiation, survival and death of neurons (44). Emerging evidence suggests that BDNF plays a

significant role in memory, and that BDNF declines as we grow older; this is believed to be one reason why memory loss and cognitive decline is often a frequent effect of ageing (45). Moreover, studies have shown that the activation of cAMP-response element-binding protein (CREB), a transcription factor that plays an important role in the formation of long-term memory (46) is positively correlated with an increase in memory after supplementation with blueberry polyphenols in aged rats (15) and an increase in spatial memory in young rats (16). It is also believed that increased cognition could be due to increased neurogenesis. Studies in animals have shown that neuron proliferation increased after blueberry supplementation (22). Recent fMRI studies have shown an increase in cerebral blood flow after supplementation with berry polyphenols, particularly in the parietal lobe and occipital lobe(32, 40). One effect of increased cerebral blood flow is an increase of oxygen and glucose to neurons, which may enhance neuronal activity.

Factors such as time-point, dosage, administration form, and choice of cognitive tasks need to be taken into consideration before recommendations can be made for future research. From the acute studies looking at time-response effects in children, it seems that there are different responses being produced at different times. For example, results have shown delayed memory performance is best performed 1.15 hours post consumption (28), whereas improvements in executive function performance is seen at 3 hrs (28, 29). The time point differences observed could be due to factors such as absorption rate, digestion, and breakdown of metabolites (43) although further testing is necessary in order to understand these mechanisms.

For chronic studies there was one case where cognitive effects for word recognition were observed at the intermediate testing point of 12 weeks but not at the final testing point of 48 weeks (41). This raises questions related to the metabolism and absorption of blueberry polyphenols, after a certain time of ingestion, and at a certain dosage. One possible explanation is that participants may have become habituated to the effect of the blueberry intervention with less cognitive benefit being evident at later stages of treatment. Furthermore, Whyte et al. (41) consider the possibility of

practice effects whereby the performance of each of participant improves over time reducing the early advantage of blueberry intervention. Nevertheless, the lack of chronic data, plus the limited range of different cognitive domains within chronic studies tested are not enough for any conclusive points to be made here.

In the papers studied, the anthocyanin content ranged from 1.35 mg (41) to 460 mg . However, higher anthocyanin concentration does not necessarily translate to better cognition compared to lower doses suggesting that a ceiling effect is likely, with higher doses producing no extra benefits. This would seem to correlate with physiological responses to blueberry intervention such as flow mediated dilation (FMD) which can be seen to peak following doses containing 766 mg anthocyanin and tail off at higher doses (10). It is believed that this improvement in endothelial function is a nitric oxide (NO) mediated response exerted by polyphenolic compounds found in blueberries.

Currently only two studies involving blueberries have investigated the effects of cerebral blood flow (CBF) where an increase in cerebral blood flow to the brain was found after acute (19) and chronic (40) blueberry treatment when compared to placebo. This suggests one mechanism by which blueberries may be exerting a positive effect on cognition. Other studies involving flavonoids and CBF have shown a similar effect, with an increase in CBF observed after an acute intake of citrus juice high in flavanones (47) or cocoa flavanols (48). This raises the question whether improved endothelial function might facilitate an increase in peripheral blood flow, and thus cerebral blood flow, which in turn may improve cognitive functioning. This is an area which still requires extensive research. Further details of studies which have considered the effects of polyphenol intervention on cardiovascular health and cerebrovascular health can be found in other reviews (for example see this recent review (49)).

In terms of the interventions themselves, there is a large variation in the actual anthocyanin content of the treatments. The flavonoid ratio in equivalent weights of the blueberry treatments also differed between studies, for example, 30g of freeze-dried blueberry powder contained 253 mg of

anthocyanins in Whyte et al. (28) whereas the equivalent fresh contained 148 mg in Whyte and Williams (27). This highlights the importance of analysing blueberry powder for polyphenol/anthocyanin content prior to starting an intervention.

In terms of study design, all but two studies (39, 45) employed a double-blind crossover, placebo-controlled design, which seems the most appropriate design when comparing a nutritional intervention against placebo. In most cases the blueberry was administered as a freeze-dried powder mixed with water and administered as a drink. Only one study mixed the powder with milk (27). Nevertheless, the evidence currently available related to the inhibition of polyphenols by dairy proteins is equivocal. Some studies demonstrate that proteins found in milk have no effect on the bioavailability of polyphenols (50) and some believe that it may affect the bioavailability of some, but not all, polyphenolic compounds (51). Ultimately it should be taken into consideration that factors other than dairy proteins may also play a role in the absorption and metabolism of polyphenols, including the gut microbiota and the chemical structure of the polyphenol (e.g hydroxyl group have a high affinity for proteins) amongst other dietary factors (52).

In conclusion, the cognitive research considering blueberry intervention currently gives an incomplete picture, with no published research as yet having considered infants, teens, young adults, or middle-aged adults. Acute effects have only been considered in children and chronic effects have only been considered in older adults. Findings from the present literature indicate that benefits might be found in most reliably in episodic memory and, under certain conditions, executive function, with the benefits for working memory at present being more equivocal. More specifically, there is a trend where improvements are seen within the executive function and episodic memory domains for children; for adults there are more memory related improvements and in adults with MCI improvements are found primarily within the episodic memory domain. Therefore, the current literature indicates that blueberry polyphenols have the capacity to improve some aspects of

cognition across certain ages, and with further investigation, is a concept which might be applied to specific real-life situations such as learning.

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Table 1. Comparison of anthocyanidin content (mg/100g FW) in different berries retrieved from USDA Database for the Flavonoid Content of Selected Foods Release 3.1 (2014)

Flavonoid subclass	Content of flavonoids in some common berries (raw form) (mg/100g of fresh weight)						
	Blueberries (highbush)	**Blackberries**	**Blackcurrant**	**Chokeberry**	**Grapes**	**Raspberries**	**Strawberries**
Anthocyanidins							
Cyanidin	8.46	99.5	62.46	344.07	1.16	45.77	1.68
Delphinidin	35.43	0.00	89.62	0.65	2.27	1.32	0.31
Malvidin	67.59	0.00	n/a	1.22	39.00	0.13	0.01
Pelargonidin	0.00	0.45	1.17	0.98	0.02	0.98	24.85
Peonidin	20.29	0.21	0.66	0.08	3.62	0.12	0.05
Petunidin	31.53	0.00	3.87	2.79	1.97	0.31	0.11
Total	**163.3**	**100.16**	**157.78**	**349.79**	**48.04**	**48.63**	**27.01**
Flavan-3-ols							
(-)-Epicatechin	0.62	4.66	0.47	n/a	0.96	3.52	0.42
(-)-Epicatechin 3-gallate	0.00	0.00	0.00	n/a	0.17	0.00	0.15
(-)-Epigallocatechin	0.66	0.10	0.00	n/a	0.08	0.46	0.78
(-)-Epigallocatechin 3-gallate	0.00	0.68	0.00	n/a	0.00	0.54	0.11
(+)-Catechin	5.29	37.06	0.70	n/a	0.82	1.31	3.11
(+)-Gallocatechin	0.12	0.00	0.00	n/a	0.00	0.00	0.03
Total	**6.69**	**42.5**	**1.17**	**n/a**	**2.03**	**5.83**	**4.6**
Flavonones							
Hesperetin	0.00	0.00	n/a	n/a	n/a	0.00	0.00
Naringenin	0.00	0.00	n/a	n/a	n/a	0.00	0.26
Total	0.00	0.00	n/a	n/a	n/a	0.00	0.26
Flavones							
Apigenin	0.00	0.00	0.00	n/a	0.00	0.00	0.00
Luteolin	0.20	0.00	0.00	n/a	1.30	0.00	0.00
Total	**0.20**	**n/a**	**0.00**	**n/a**	**1.30**	**0.00**	**0.26**
Flavonols							
Isorhamnetin	n/a	n/a	0.12	n/a	n/a	0.00	0.00
Kaempferol	1.66	0.27	0.71	0.34	0.00	0.06	0.50
Myricetin	1.30	0.67	6.18	0.00	0.01	0.00	0.04
Quercetin	7.67	3.58	4.45	18.53	1.04	1.05	1.11
Total	**10.63**	**4.52**	**11.46**	**18.87**	**1.05**	**1.11**	**1.65**

Table 2: Key studies investigating the effects of blueberry supplementation on cognition in healthy children.

Author(s)	Treatment	Total Anthocyanin (mg)	Total Polyphenol (mg)	Study design	Study type	Measurement time points	Size (n)	Age range (years)	Cognitive tests (cognitive domain tested in parenthesis)	Key findings	Effect size (Cohen's d)
Whyte & Williams (2015)(27)	200g fresh BB	**143**	N/A	Double blind, placebo-controlled crossover design	Acute	2 hrs	14	8-10	**(1)** Go-NoGo (EF) **(2)** Stroop (EF) **(3)** Rey's Auditory Verbal Learning Task (AVLT) (EM) **(4)** Object Location Task (EM) **(5)** Visual N-back (WM)	AVLT: Delayed Recall • BB vs Placebo (p= .038) AVLT: Proactive Interference • BB vs Placebo (p= .043*) *Better placebo performance.	0.904 0.883
Whyte, Schafer & Williams (2016)(28)	Freeze dried BB powder **(1)** 15 g (120 g FE) **(2)** 30g (240 g FE)	**(1) 127** **(2) 253**	N/A	Double blind, placebo-controlled crossover design	Acute	1.15, 3, & 6 hrs	21	8-10	**(1)** Auditory-Verbal Learning Task (EM) **(2)** Modified Flanker Task (MFT) (EF) **(3)** Go-NoGo (EF) **(4)** Picture Matching Task (PMT) (EF)	AVLT: Final Acquisition at 1.15hr • 30g BB vs Placebo (p=.023) AVLT: Delayed Word Recognition at 6hrs • 15g BB vs Placebo (p=.038) MFT: Incongruent Trial Accuracy at 3hrs • BB vs Placebo (p=.035)	0.908*** 0.245 0.201
Whyte, Schafer & Williams (2017)(29)	Freeze dried BB powder, 30g (240 g FE)	253	N/A	Double blind, placebo-controlled crossover design	Acute	3 hrs	21	7-10	**(1)** Modified Attention Network Task (MANT) (EF)	MANT: Reaction Time • BB vs Placebo (p = .048)	0.940***
Barfoot et al (2018)	Freeze dried BB powder, 30g (240 g FE)	253	N/A	Randomised, single-blind, parallel-groups design	Acute	2 hrs	54	7-10	**(1)** Rey's Auditory-Verbal Learning Task (EM) **(2)** Modified Attention Network Task (MANT) (EF) **(3)** Test of Word Reading Efficiency-2; TOWRE-2 (reading efficiency)	AVLT: Total acquisition performance • BB vs Placebo (p= 0.035) AVLT: Short delay recall • BB vs Placebo (p=0.04) MANT: Reaction time • BB vs Placebo (p=0.018).	0.425 0.405 0.175

Note. BB = Blueberry, FE = Fresh Equivalent; EM = Episodic Memory, EF = Executive Function, WM = Working Memory, PF = Psychomotor Function.

Table 3: Key studies investigating the effects of blueberry supplementation on cognition in healthy adults.

Author(s)	Treatment and amount	Total Anthocyanin (mg)	Total Polyphenol (mg)	Study design	Study type	Measurement time points	Size (n)	Age range (years)	Cognitive tests (cognitive domain tested in parenthesis)	Key findings	Effect size (Cohen's d)
Schrager et al. (2015)(36)	Frozen BB - 2 cups daily (approx. 300g FE).	N/A	N/A	Placebo controlled, parallel design. Randomisation and blinding not stated.	Chronic	Baseline and week 6	20	61-80	**(1)** Simple Reaction Time (PF) **(2)** Trail Making Test part B (EF) **(3)** Dual-Task adaptive gait test (DTAG) (EF/PF)	DTAG: Step Errors • BB vs Placebo (p = .048)	1.16**
Miller et al . (2017)(35)	Freeze dried BB powder, 24g (approx. 150g FE)	460	864	Double-blind, placebo-controlled, parallel design	Chronic	Baseline, day 45 and day 90.	37	60-75	**(1)** Task-switching test (TST) (EF) **(2)** Trail-making test (TMT) (EF) **(3)** California Verbal Learning Test. CVLT-II) (EM) **(4)** Digit span task (DS) (WM) **(5)** Virtual Morris Water Maze (vMWM) (WM) **(6)** Attention Network Task (ANT) (WM)	TST: Switch Cost • BB improvement across visits (p = .033) CVLT: Repetition Errors • BB improvement across visits (p = .031)	0.629*** 0.759***
Bowtell et al. (2017)(40)	BB concentrate, 30ml (FE N/A)	387	N/A	Double blind, placebo-controlled crossover design	Chronic	Baseline and week 12	26	60-75	**(1)** Detection task (PF) **(2)** Groton maze timed chase test (EF) **(3)** Groton maze learning test (EF) **(4)** identification task (EF) **(5)** International shopping list task (EM) **(6)** 1-back and 2-back memory tasks (WM)	2-back task • BB vs Placebo (p = .05 trend) fMRI: Brain activity • BB vs Placebo (p < .001)	n/a

Whyte et al. (2018)(41)	**(1)** 500 mg Wild BB Powder (WBP500) **(2)** 1000 mg Wild BB Powder (WBP1000) **(3)** 111 mg Wild BB Extract (WBE111)	**(1)** 1.35 **(2)** 2.7 **(3)** 7	**(1)** 35 **(2)** 70 **(3)** 50	Double blinded, placebo-controlled parallel design	Chronic	Baseline, week 12 and week 24	112	65–80	**(1)** RAVLT (EM) **(2)** Object recognition task (EM) **(3)** Corsi Blocks task (EM) **(4)** Serial subtractions tasks (WM) **(5)** The Sternberg memory scanning task (WM) **(6)** Modified Attention Network Task (EF) **(7)** Stroop (EF)	RAVLT: Word Recognition at 12 weeks • WBE111 vs Placebo (p = .038) Corsi Blocks: Total Sequences • WBE111 vs Placebo (p = .069 trend)	0.578 0.289

Note. BB = Blueberry, FE = Fresh Equivalent; EM = Episodic Memory, EF = Executive Function, WM = Working Memory, PF = Psychomotor Function.

Table 4: Key studies investigating the effects of blueberry supplementation on cognition in adults with MCI.

Author(s)	Treatment	Total Anthocyanin (mg)	Total Polyphenol (mg)	Study design	Study type	Measurement time points	Size (n)	Age range (years)	Cognitive tests (cognitive domain tested in parenthesis)	Key findings	Effect size (Cohen's d)
Krikorian et al . (2010)(33)	Wild BB juice. By participant weight: **(1)** 54-64 kg = 444 ml **(2)** 65–76 kg = 532 ml **(3)** 77–91 kg = 621 ml (FE N/A)	**(1)** 428 **(2)** 512 **(3)** 598	**(1)** 1056 **(2)** 1266 **(3)** 1478	Double blind, placebo-controlled crossover design	Chronic	Baseline and at 12 weeks	9	Mean age: 72	**(1)** California Verbal Learning Test (CVLT) (EM) **(2)** Verbal paired associate learning test (V-PAL) (EM)	V-PAL: Cumulative Learning • BB improvement across visits (p = .009) • BB vs Placebo (p = .03) CVLT: Word Recall • BB improvement across visits (p = .04)	1.78* 0.96** 1.18*
Boespflug et al . (2017)(32)	25 g Freeze-dried BB powder (approx. 150g FE)	269	417	Double blind, placebo-controlled crossover design	Chronic	Baseline and at 16 Weeks	16	68 –92	**(1)** n-back WM task (WM)	No sig. effects after 16 weeks of supplementation. fMRI: Brain activity • BB relative to baseline (p < .01)	1.82 (left inferior parietal gyrus) and 1.94 (left pre-central gyrus)

McNamara et al . (2018)(34)	25 g Freeze-dried BB powder (approx. 150g FE)	269	417	Double blind, placebo-controlled crossover design	Chronic	Baseline and at 24 Weeks	19	62-80	**(1)** Dysexecutive Questionnaire (DEX) (EF/WM) **(2)** Trail making test A and Trail making test B (EF) **(3)** Controlled Oral Word Production (EF) **4)** Hopkins Verbal Learning Test (HVLT) (EM)	DEX: Cognitive Symptoms • BB vs Placebo (p = .05) HVLT: Memory Discrimination • BB vs Placebo (p = .04)	0.68** 0.68**

Note. BB = Blueberry, FE = Fresh Equivalent; EM = Episodic Memory, EF = Executive Function, WM = Working Memory, PF = Psychomotor Function

*= Effect sizes reported from article (Cohen's *d*)

**= Effect sizes originally reported from article as Cohen's *f*, and converted to Cohen's d for the purpose of this review.

***=Effects sizes originally reported as partial ETA square and converted to Cohen's d for the purpose of this review.