REVIEW

Food-based anthocyanin intake and cognitive outcomes in human intervention trials: a systematic review

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anthocyanins, cognition, flavonoids, polyphenols.

Abstract

Background: Preclinical evidence suggests that the anthocyanins, which comprise a subclass of dietary flavonoids providing the purple and red pigmentation in plant-based foods, may have a beneficial impact on cognitive outcomes.

Methods: A systematic review was conducted to identify the published literature on food-based anthocyanin consumption and cognitive outcomes in human intervention trials. The literature search followed PRISMA guidelines and included six databases, as well as additional hand searching.

Results: Seven studies were included in this review, comprising acute trials (n = 4) and longer-term (n = 3) interventions that assessed multiple cognitive outcomes in children, adults and older adults with cognitive impairment. Six of seven studies reported improvements in either a single, or multiple, cognitive outcomes, including verbal learning and memory, after anthocyanin-rich food consumption. As a result of methodological limitations and the large clinical and methodological diversity of the studies, the pooling of data for quantitative analysis was not feasible.

Conclusions: The impact of food-based anthocyanin consumption on both acute and long-term cognition appears promising. However, adequately powered studies that include sensitive cognitive tasks are needed to confirm these findings and allow the translation of research into dietary messages.

Introduction

The rapidly ageing population has led to a major projected increase in the prevalence of neurodegenerative diseases such as dementia (1), which has resulted in intense research interest regarding the role of diet in neuroprotection. Dietary approaches are especially considered to comprise safe and effective methods for the prevention of cognitive disorders and the maintenance of cognitive function. The consumption of dietary flavonoids was shown to both enhance cognitive function (2) and prevent age-related cognitive decline in older adults (3). The neuroprotective effects of flavonoids have been well-documented (4-8), although most of the research has focused on animal models, limiting translation to humans who utilise more complex cognitive domains, such as executive functioning. Because of their strong antioxidant potential (9), it has been hypothesised that flavonoids limit neurodegeneration by reducing neuro-inflammation and protecting the cellular architecture in the brain (10). Flavonoids may also contribute to improved cognitive function by increasing the number and strength of neuronal signals (11) via increased brain blood flow (7) and an ability to initiate neurogenesis (5) in areas of the brain associated with learning and memory. A large proportion of this research has specifically highlighted the benefits of anthocyanin-rich foods (12-15).
Anthocyanins are a subclass of dietary flavonoids (i.e. different from proanthocyanidins, which are polymers of flavanols), which provide the purple, red and blue pigmentation in plant-based foods (16). Typical food sources of anthocyanins are fruits such as apples, grapes, plums; berries such as strawberries, blueberries and blackberries; vegetables such as red cabbage, red onion and eggplant; and beverages such as red wine and red grape juice (17). Approximately 640 individual anthocyanins have been identified to date (18), however, anthocyanins naturally occur most ubiquitously in six common aglycones (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, malvidin) in various glycosylated and acylated forms (18).

Anthocyanins are unique among flavonoids as a result of their ability to reversibly change their structures in response to changing pH values, from the stable red to orange coloured flavylum cation at pH < 2 to the colourless and less stable carbinol and chalcone forms at higher or neutral pH (18–20). This unique feature may have an impact on their stability and binding ability (e.g. to enzymes) throughout the gut, which has a dramatically changing pH environment (‘acid’ stomach to ‘neutral’ intestine).

The metabolism and absorption of anthocyanins has been documented previously (21). Briefly, food-based anthocyanins are consumed and are either quickly absorbed as intact molecules via the stomach, or after extensive metabolism in the small and large intestine or the colon, to enter the circulation as metabolites such as phenolic acids (21). In previous animal and human studies, only very low amounts of intact (non-metabolised) anthocyanin glycosides could be detected in plasma and urine after ingestion of pure anthocyanin compounds and/or anthocyanin-rich food and beverages; 15 animal and 17 human bioavailability studies were comprehensively reviewed by Pojer et al. (19). However, recent human studies have clearly demonstrated that dietary anthocyanins undergo intensive metabolism after consumption and that those metabolites are the main transport forms in vivo (22–25). For example, thirty-two anthocyanin-derived metabolites could be detected in the urine, serum and faeces of eight healthy volunteers after the consumption of 500 mg of 13C-labelled cyanidin-3-glucoside (25). In another study published by Ludwig et al. (22), 17 metabolites were quantified in urine collected from nine volunteers after the consumption of 300 g of blended raspberries (22). Previously, the bioavailability of anthocyanins was considered to be very low (urinary recoveries <2% of intake in most studies), although it is now estimated as being at least 15% if detectable in vivo metabolites are also taken into account (22).

The main biological activity of anthocyanins in vivo was assumed to be their antioxidant capacity and protection against lipid peroxidation (26). However, recent studies have identified more complex molecular effects of anthocyanins on signalling pathways in cells that may play a significant role in their protection against chronic diseases (19,26,27). Modulation of neuronal functions by anthocyanin-rich food has been reported in several animal studies (19). It is suggested that these neuroprotective effects (e.g. improved memory, learning, cognitive and motor functions) by anthocyanins (and/or their in vivo metabolites) are mediated via inhibition/suppression of interleukin-1β, tumor necrosis factor-α and nuclear factor kappa B. The modulatory effects of anthocyanins on important structural and synaptic plasticity markers have also been reported. Acute improvements in cognition after consuming anthocyanin-rich foods have been associated with increased cerebral blood flow (1–2 h and 6 h after consuming anthocyanin-rich blueberries) and improvements in endothelium-dependent vasodilation (as assessed by flow-mediated dilatation) (28,29).

A body of evidence concerning the benefits of anthocyanin consumption in human health, including protective effects against age-related neurodegeneration and cognitive decline, has accumulated over the recent years (16,19). Animal studies have provided evidence that anthocyanin-rich food consumption can improve several cognitive functions, including long-term memory (30), spatial-working memory (31) and object-recognition memory (32). In addition, improvements in motor performance (i.e. balance and coordination) have been documented after receiving an anthocyanin-rich blueberry supplemented diet in an animal model (14). Intact (non-metabolised) anthocyanins were detected in the brains of rats as soon as 10 min after their consumption, and these concentrations were found to be correlated with improvements in learning and memory tasks (13). Of particular interest, supplementation of the diet with anthocyanin-rich fruits, such as blueberries and strawberries, was shown to not only maintain, but also reverse age-related cognitive decline in animal models (11,33,34). A large epidemiological study of 16 010 older women enrolled in the Nurses’ Health Study reported significant associations between anthocyanin-rich blueberry and strawberry consumption and the maintenance of cognitive function (a composite score of six cognitive tests, including various measures of memory) over 4 years (35). It was reported that those individuals who consumed the highest amount of berries had delayed cognitive ageing by up to 2.5 years compared to those who ate the least (35). Despite their known health benefits, anthocyanins are often overlooked in human intervention trials that investigate the impact of overall flavonoid intake on cognitive outcomes (36). In a 2009 systematic review of human
intervention trials assessing the impact of flavonoids on cognitive outcomes (36), no studies related to anthocyanins were included.

There has been increasing interest in other areas of anthocyanin research, with a 15-fold rise in the number of published papers between 1990 and 2013 including the word ‘anthocyanin’ in their title and abstract (37). Important advances in an increased understanding of the bioavailability and metabolism of anthocyanins (e.g. bio-transformation by the gut microbiota), coupled with a greater focus on the role of plant foods in brain health (e.g. neuroprotective activities), warrant a targeted review aiming to summarise the evidence to date on human trials on anthocyanins and cognitive function.

Materials and methods

A systematic review of the literature was performed aiming to summarise the published literature on the effects of food-based anthocyanin consumption on cognitive function in human trials. The review also aimed to identify gaps in the literature and to consider the implications for future study designs. The search was conducted using methodology outlined in the PRISMA statement (38), according to the search strategy outlined below.

Types of studies

Studies were eligible for inclusion in this review if they:

- were randomised or nonrandomised controlled trials; cross-over trials (excluding case studies and observational studies)
- examined humans (any age, sex);
- administered a naturally occurring anthocyanin containing food or beverage (the anthocyanin content of the food needed to be objectively measured; foods fortified with additional anthocyanin were excluded);
- measured change in cognitive function using single or multiple instruments that had previously been validated in a similar population; and
- had full text available.

Only studies written in the English language were considered because of a lack of translational resources, although studies from any country were eligible if they met the rest of the inclusion criteria.

Search terms

The following search terms were developed:

- Anthocyan* and/or
- Flavonoid* and
- Cognit* and/or
- Memory

The * indicates a truncation, to ensure all variations of the search terms were included.

Databases

A three-stage search was conducted. First, a search of the following databases (abstract, title and keywords) was performed using the search terms using the Boolean terms AND/OR as appropriate:

- Scopus (1960 to Oct 2015)
- Medline (1960 to Oct 2015)
- CIIHAINAL (1960 to Oct 2015)
- Psychology and Behavioural Sciences Collection (1960 to October 2015)
- Psyclnfo (1860 to October 2015)
- Web of Science (1965 to October 2015)

Second, hand searching of the reference list of identified studies was conducted. Third, an internet search was performed to identify any published studies outside the realms of the selected databases. The records were exported from the databases and managed in ENDNOTE X4 (Thomson Reuters, New York, NY, USA). After screening and selection of studies according to the inclusion criteria (Fig. 1), relevant summary data were extracted into a template made for this purpose, including the study design, intervention, dose, methods and results.

Results

The search returned 1684 articles and four other potential studies were identified through hand-sorting of reference lists. After duplicates were removed and the titles screened, 35 potential studies were identified. After screening abstracts, a further 25 were excluded and, after reading the full text articles of 10 studies, a further three were excluded according to the inclusion/exclusion criteria (Table 1), leaving seven papers in the final review (Fig. 1).

Description of studies

Statistical pooling of the data from the seven studies considered eligible for the review (39–45) was not possible because of the large clinical and methodological diversity of the studies. This heterogeneity resulted from variability in the characteristics of the participants and type of intervention (including food sources and dose of anthocyanins), varying study designs and study duration, as well as differences in choice of cognitive outcomes. The findings are therefore presented in narrative form. The characteristics and relevant information of each study are described in Table 2. Publication dates ranged from 2010 to 2015. Of the seven included studies,
three were randomised, double-blind, placebo-controlled studies (39,40) that assessed the daily consumption of anthocyanin-rich fruit juices on cognitive outcomes. The remaining four were placebo-controlled cross-over trials, either randomised (n = 2) (42,44) or nonrandomised (n = 2) (41,45) that assessed the acute impact of anthocyanin rich fruit juices on cognitive outcomes. Two studies investigated older adults with mild cognitive impairment (39,40), one study included older adults with dementia (43), one study was conducted in young, healthy adults (42), one study included a combination of older adults with and without dementia as well as healthy younger adults (44), and two studies were undertaken in children (41,45). The studies were undertaken in New Zealand (42), the USA (39,40), the UK (41,45) and Australia (43,44).

Description of the interventions

In all seven studies, the vehicle for anthocyanin administration was a beverage. Four studies utilised a preformulated juice (39,40,43,44), one study (42) utilised a juice processed from fresh fruit (150 g of fruit yielded approximately 140 mL of juice), two studies used a powdered freeze-dried fruit made up with water (42) or cordial (45) and one study (41) blended fresh fruit into a smoothie. Two studies based the beverage serving size on the body weight of each participant (39,40) providing between 6 and 9 mL of juice kg⁻¹ body weight of juice (providing between 428 and 598 mg anthocyanins day⁻¹ divided over three servings). One study (42) altered the serving sizes of the juice to consistently deliver a mean (SD) of 525 (5) mg of polyphenols per 60 kg of bodyweight per participant. Four studies (41,43–45) administered a standard serving size to each participant, regardless of body size, providing total anthocyanins of between 55 mg (44) and 253 mg day⁻¹ (45).

Outcome assessment

Cognitive outcomes were measured using valid, referenced instruments in all studies. Four studies measured acute change in cognition (41,42,44,45), whereas the other three studies measured longer-term change in cognition of between 12 and 16 weeks (39,40,43). Detailed results, including significant effects, are reported below.

Longer-term anthocyanin supplementation and cognition

Two of the longer-term studies evaluated verbal learning and memory in older adults with mild cognitive impairments (39,40) using the California Verbal Learning Test and one study measured the same outcome using the Rey Auditory Verbal Learning Test (RAVLT) (43). One study
measured change in explicit episodic memory using the Paired Associate Learning Test (40) and two studies assessed change in mood using the Geriatric Depression Scale (39,43). One study measured several other cognitive domains, including working memory (self-ordered pointing task), semantic memory (the Boston naming test), executive function (trail making test, category and letter verbal fluency) and short-term memory (digit span backwards task) (43). One study (39) measured brain activity objectively, using functional magnetic resonance imaging (fMRI) brain imaging, which indicates stimulation and activity in specific regions of the brain.

Krikorian et al. (39) reported that, after 16 weeks of supplementation with concord grape juice, there was no between-group difference for performance on the CVLT learning task, although there was a reduction in interference on the CVLT memory task compared to baseline ($P = 0.04$). There was a nonsignificant effect on mood. Analysis of the fMRI brain analysis indicated that there was greater activation in the anterior and posterior regions of the brain for the intervention group only (39). In another study, the same research team of Krikorian et al. (40) reported significant improvements on the verbal paired associate learning task ($P = 0.009$) after 12 weeks of supplementation with blueberry juice. However, both of these studies had small sample sizes of only 10 in the Concord grape juice intervention arm (39) and nine in the blueberry juice intervention arm (40).

Kent et al. (43) conducted a larger trial ($n = 21$ per group) in older adults with mild–moderate dementia and reported that, after 12 weeks of supplementation with cherry juice (138 mg day$^{-1}$), there were significant improvements in verbal fluency ($P = 0.014$), short-term memory (RAVLT) ($P = 0.014$) and long-term memory (RAVLT delayed) ($P \leq 0.001$).

### Acute anthocyanin supplementation and cognition

The four acute feeding studies evaluated change in cognition using a variety of tests: the digital vigilance test (a measure of attention) (42), the rapid visual information processing test (attention and working memory) (42), the go-no-go test (response inhibition) (41,45), an objective location task (spatial memory) (41), a visual-n back test (attention and short-term memory) (41), a task switching test (higher executive function) (44), a pattern and letter comparison task (speed of processing) (44), a picture matching task (processing and response interference) (45) and the auditory verbal learning task (verbal learning and memory) (45). Two studies used the Stroop test to measure attention and inhibition (41,42) and the RAVLT to measure verbal learning and memory (41,44).

Watson et al. (42) compared three conditions (placebo versus powdered blackcurrant extract with 7.7 mg anthocyanins kg$^{-1}$ versus blackcurrant juice with 8 mg kg$^{-1}$, based on body weight) on acute cognition in 36 young adults by administering tasks seven consecutive times over 65 min after consumption of the beverages. Significant improvements in accuracy were reported, as measured by the rapid visual information processing test ($P = 0.011$), when participants consumed the blackcurrant extract versus placebo, as well as improvements in reaction time for some repetitions (1, 4 and 7) on the digital vigilance task compared to baseline tests.

<table>
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<th>Study reference</th>
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<td>10 (74)</td>
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<td>11 (75)</td>
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<td>Editorial</td>
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<td>Longer-term</td>
<td>Krikorian et al. (39)</td>
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<tr>
<td>Krikorian et al. (40)</td>
<td>9 older adults [5 men, mean (SD) age 76.2 (5.2) years] mild cognitive impairment (clinical dementia rating)</td>
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<td>Kent et al. (43)</td>
<td>42 older adults, 70+ years, mild-moderate dementia (geriatrician diagnosed)</td>
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<tr>
<td>Watson et al. (42)</td>
<td>36 young adults [mean (SD) age 24.8 (3.9) years]</td>
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<td>Whyte et al. (45)</td>
<td>21 children [9 male, mean (SD) age 8.7 (0.67) years]</td>
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<td>Participants</td>
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<tr>
<td>Whyte et al. (41)</td>
<td>16 children [10 male, mean (SD) age 9.17 (0.6) years]</td>
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<tr>
<td>Caldwell et al. (44)</td>
<td>16 adults: young adults, n = 6 [mean (SD) age 21.8 (0.97) years]; older adults, n = 5 [mean (SD) age 74.1 (7.9) years]; five older adults with dementia (geriatrician diagnosed), [mean (SD) age 79.8 (3.6) years]</td>
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AVLT, auditory verbal learning task; fMRI, functional magnetic resonance imaging; HPLC, high-performance liquid chromatography; ORAC, oxygen radical absorbance capacity; RAVLT, Rey Auditory verbal learning test; RVIP, rapid visual information processing
The aim of this systematic review was to assess the effects of consumption of anthocyanins present in foods, without supplementing (and therefore not giving isolated anthocyanins) on domains of cognitive function, as well as to highlight gaps in the literature and address the applicability of the available evidence for development of future study designs. Despite more than a decade of preclinical evidence supporting the association of anthocyanin intake on cognitive outcomes, there remains a lack of experimental studies conducted in humans. Six of the seven studies included in the review reported positive benefits of anthocyanin consumption on some aspect of cognition; however, the generalisability of the findings to other clinical or healthy populations is limited by the small study sample sizes and inconsistent methodologies. There was a lack of homogeneity in the study designs that prevented pooling of data for a quantitative analysis of the magnitude of effect. From the available data, it is unclear in which population the effects are most likely or which doses of anthocyanins and/or their food sources are effective at inducing cognitive benefit.

The cognitive domains that appear to be acutely sensitive to consumption of anthocyanin-rich foods are verbal learning and memory, whereas, additionally, longer-term consumption also impacts upon attention and working memory. However, there is a lack of consensus regarding the choice of best instruments and methods to employ in studies that investigate potential change in cognitive function. The seven identified studies used a wide range of tools to address multiple cognitive domains between studies. This limitation has been recognised and evaluated in a recent methodological review. That review concluded that future studies should be guided by positive findings from previous similar research, and that it may be best to continue to include a battery of sensitive and specific tools that address multiple cognitive domains, until there is better understanding of specific brain functions that are influenced by these bioactive components in foods.

The duration of time needed to see an effect within both acute and longer-term trials is also poorly understood. The studies of Kent et al. and Krikorian et al. suggest that cognitive benefits are seen after 12 weeks of intervention, and that extending the study period to 16 weeks may not necessarily improve the strength of the association. The timing related to the acute impact of food-based anthocyanins on cognition is hampered by the limited knowledge of whether the intact anthocyanins or their metabolites, or both, are responsible for the beneficial effects of an anthocyanin-rich diet. Two of the acute studies included in this review did not extend their trial times to encompass the secondary uptake of anthocyanin-related metabolites in the colon, and thus may have missed an important later interaction that these compounds have on cognition. The acute cross-over trial by Caldwell et al. found no significant change in cognition 6 h post-intervention but the small numbers of participants suggest that it was likely to be underpowered to observe cognitive effects.

Another outcome from this systematic literature review is that biomarker data to confirm anthocyanin uptake in the included studies were lacking. Only one study conducted an objective analysis of biological specimens to determine the uptake and metabolism of the anthocyanins present in the intervention foods. Such fundamental data are needed to confirm the bioavailability of anthocyanins from different foods and to determine which of the bioactive components are specifically associated with the observed health benefits. There is growing evidence to suggest that the biological activity of some flavonoids may improve during metabolism, in
comparison with their intact variants, which further complicates the interpretation of their biological effects of both the type and quantity found naturally in foods. In addition to metabolic conjugation and methylation of flavonoids in the human body, intestinal bacteria are also considered to play an integral role in their metabolism (48). Studies that investigate the health effects of anthocyanins will need to consider new paradigms to understand their bioactivity in vitro, in an attempt to better explain their mechanisms of action (48). A recent study (23) attempted to account for the fate of the remaining 99% of ingested anthocyanins that are not recovered in blood and urine. Rather than confirm that anthocyanins had low bioavailability resulting from poor absorption and chemical instability (48), the study identified an extensive biotransformation of anthocyanins that resulted in a relative bioavailability (i.e. the amount absorbed and found in blood, urine and breath) of 12%, rather than the previously assumed 1%. An extensive diversity of metabolites was identified (>30 unique structures), including phenolic, hippuric, phenylacetic and phenylpropenoic acids. These findings have important ramifications for future studies, in which urine and plasma sampling periods in short-term bioavailability studies should be extended because anthocyanin-derived metabolites are still being excreted up to 48 h post-ingestion (22,23,25). As well as plasma/serum and urine, faeces should also be collected as an integral sampling ‘matrix’ in future human research to measure the metabolites produced by the gut/colic microbiota. This information will be essential to further clarify how dietary anthocyanins exert their biological activities in vivo.

In the case of the full complement of flavonoid metabolites not being able to be measured, other characteristic biomarkers of intake could be identified and assessed (49). For example, one study measured change in nonspecific measures of inflammation (43) as a potential reflection of the anti-inflammatory mechanisms related to flavonoid bioactivity, and reported no significant impact on C-reactive protein or interleukin-6 levels. However, the various other mechanisms by which flavonoids are purported to provide neuroprotection and improve memory and cognition (5,6) are not explained by anti-inflammatory biomarkers, and thus this choice of biomarker may not necessarily reflect the total bioactivities of flavonoids in vivo.

Ageing is associated with changes in the functional properties of the digestive system, such as delayed transit time, losses in absorptive function and the influence of prescription medication use on gut microbiota (50). Therefore, the effect of age may influence the bioavailability and metabolism of anthocyanins (50). This hypothesis has not been adequately tested but should be a consideration when comparing interventions across age groups. The studies included in the present review investigated groups ranging in age from children (8–10 years) to older adults (>68 years) with mild-cognitive impairment and dementia. Disease states that affect brain function, including vascular and nonvascular dementia, as well as Alzheimer’s disease, may also result in different measureable outcomes in these groups (51), as shown by the limited impact of antioxidant clinical trials in these groups (51). It is therefore important to clearly define patient groups and describe the differences in outcomes between children, as well as healthy young and older adults.

The vehicle for provision of anthocyanins also warrants consideration. All of the studies in the review provided anthocyanins in the form of a beverage, although the use of this vehicle was not well explained. It can be speculated that the provision of a preformulated juice is an ideal way to control the variation of anthocyanins in the fresh-fruit equivalent, which can be heavily influenced by growth conditions and can differ between seasons. Provision of a juice also reduces the impact of fruit seasonality on having sufficient fruit available year-round. The impact of food processing can also substantially alter the anthocyanin content of foods, especially with regard to heating or cooking. For example, heating of dark anthocyanin-rich blood plums during jam making resulted in losses of up to 70% of their total anthocyanin content (52). Additionally, some studies administered relatively large doses of anthocyanin-rich beverages (up to over 600 mL), presumably to maximise the potential of the bioactive anthocyanins. The division of the anthocyanin-rich food over three servings per day in the longer-term trials (39,40) may have diminished their bioactivity, where the provision of smaller doses may not have provided a sufficient concentration of anthocyanin to exert a physiological effect, compared to an increased bioactivity if a larger serve is consumed at once. There is a trade-off in clinical trial methodology between reflecting the usual intake of anthocyanin-rich foods, as spread throughout the day, and ensuring that a sufficiently high dose is provided (in a single serving) to observe outcomes. The dose–response mechanisms for the impact of anthocyanin-rich food on cognition have not been well investigated, and the differences in anthocyanin intake across the intervention studies were vast. One study (44) that provided a juice much lower in anthocyanin content (18.6 mg/100 mL; 55 mg per serving) than the other trials reported no acute cognitive benefits, and therefore this dose may not be sufficiently high to induce acute cognitive change. Future trials are needed to further clarify the dose-dependent responses associated with the consumption of anthocyanin-rich foods. Interestingly, the
cognitive benefits associated with anthocyanin-rich food consumption do not appear to be limited to one food, with benefits documented after consuming blueberries, blackberries and cherries.

Berries, particularly blueberries and blackberries, have received the most interest in studies investigating the health impacts of anthocyanin-rich foods. Many other anthocyanin-rich sources have been overlooked, including vegetables such as red onions and cabbage. The potential for these foods to influence cognition needs to be confirmed because the large dependence in research on berries limits the translation of the outcomes to nutritional advice for the population. The consumption of large amounts of berries is not always feasible because berries are often expensive, seasonal (not available year-round) and can spoil quickly. The use of freeze-dried fruits to overcome these barriers may be a possibility, providing that the anthocyanin content of the fresh fruit equivalent is not spoiled. Freeze-dried fruit may be a nutritionally more preferable way in which to consume anthocyanins compared to the encapsulated anthocyanins or anthocyanins added to other processed foods. This is because anthocyanin pigments present within natural foods are unlikely to be working independently because plants typically contain a complex mixture of flavonoids that have synergistic bioactivities (53). Some studies have found that anthocyanin bioactivity may be increased when they are consumed within a mixture of polyphenols (e.g. as they would naturally occur within a complex food matrix) (54). This emphasises the importance of investigating whole food sources of anthocyanins, rather than isolated and encapsulated anthocyanins when studying health-related outcomes.

Background diet was considered by only one of the intervention studies (45), where recommendations were provided on which foods to avoid the evening prior to testing day. Diet was controlled on the day of the intervention in the longer acute trials (44,45) but was not controlled in the longer-term trials. Controlling the background diet, or at least monitoring it, is an important consideration in flavonoid trials (49) with respect to isolating the effect of the intervention. The provision of low flavonoid and/or washout diets before and during trials may be an effective way of reducing the impact of habitual diet on intervention outcomes, although monitoring habitual diet throughout the period of longer-term interventions may also be useful for identifying potential dietary confounders (49). Alternatively, excluding potential participants with abnormally high (vegans, vegetarians) or low flavonoid intake may be an easier way of reducing the influence of background diet on the study results (49).

The potential benefit of an anthocyanin intervention to improve cognitive outcomes when the background diet of an individual is already rich in dietary flavonoids and/or anthocyanins needs to be considered in future research. Ideally, an intervention would contain an anthocyanin dosage that can feasibly be achieved in a habitual diet. The ideal protocol for standardising the background diet among participants, either by providing a controlled diet or providing recommendations regarding the intake of flavonoid rich foods, including the length of run-in periods in flavonoid trials, has not been investigated and identified, although a minimum of 3 days has been suggested (49). However, manipulation of the background diet of participants limits the usefulness of the intervention findings in a real world setting.

The implication of study design (i.e. a cross-over design versus parallel groups) is important when considering flavonoid trials. As a result of the high inter-individual variability in the metabolism of flavonoids (55), a cross-over design where each individual serves as their own control is arguably more appropriate, especially in trials with small sample sizes. In these study designs, however, the influence of learned practice effects on performance in the cognitive tasks associated with repeated testing would need to be accounted for.

Three studies were excluded on the basis of not having a measure of anthocyanin content of the intervention juice (56-58). Hendrickson et al. (58) measured total phenolic content but did not report anthocyanin content specifically. Previous research has shown that both cranberry (59) and grape juice (39) contain anthocyanins but, without an objective measure of their anthocyanin content, the extent to which their bioactivity relates to the anthocyanin content, specifically, cannot be speculated. These studies showed that, in older adults (n=5), daily concord grape juice consumed (4–6 mL kg⁻¹ body weight divided over three servings per day) over 12 weeks was associated with significant improvements (P = 0.04) in verbal learning and memory on the California Verbal Learning Test (56). In young adults (n=35), grape juice (10 mL kg⁻¹ body weight) had no impact on acute cognition (58) (implicit memory as measured by a word fragment completion task) and cranberry juice consumption (470 mL consumed twice per day) had no significant impact on cognitive outcomes (57).

Lastly, it is important to note here the distinction between the terms anthocyanins and anthocyanidins, which are sometimes used interchangeably but incorrectly in the literature. Anthocyanins are glycosylated anthocyanidins (aglycones), which are the naturally occurring water-soluble pigments in coloured fruits and vegetables, whereas anthocyanidins are rarely found in nature because of their poor stability (18,19) and differ in terms of molecular size and polarity (18). Anthocyanins are more water soluble, whereas anthocyanidins are more hydrophobic (18,19). Anthocyanidins are the initial
degradation products in the gut microbial metabolism of anthocyanins, which can be transported to the colon as matrix-bound glycosides (60). After hydrolysis by microbiota, the released and unstable aglycones are rapidly degraded to their respective phenolic acids depending on their aglycone structure (e.g. cyanidin to protocatechuic acid, malvidin to syringic acid and peonidin to vanillic acid) (60). There are contradicting reports in the literature about the (bio)activity of anthocyanins and anthocyanidins. Tsuda et al. (61) measured a stronger in vitro antioxidative activity of cyanidin (anthocyanidin) than cyanidin-3-glucoside (anthocyanin) in an experimental liposome/rabbit erythrocyte membrane model. However, a similar in vitro radical scavenging activity was reported for eight common anthocyanidins and seven anthocyanins (62), whereas a weaker inhibitory effect on cell transformation could be observed for delphinidin glycosides (anthocyanins) compared to their sugar-free aglycone delphinidin (anthocyanidin) (63). However, it is unlikely that intact (non-metabolised) anthocyanidins exert any significant biological effects because of their instability at neutral and physiological pH conditions (64,65).

In conclusion, the impact of food-based anthocyanin consumption on both acute and long-term cognition appears to be promising, with six of seven studies reporting improvements in either single, or multiple, cognitive outcomes after anthocyanin-rich food consumption. However, adequately powered studies that utilise sensitive cognitive tasks previously shown to detect changes in cognition in anthocyanin trials (e.g. the RAVLT for measuring change in verbal learning and memory) are needed to confirm these findings. Future research should focus on follow current recommendations for designing, implementing and reporting of flavonoid trials (49). Studies should focus on clarifying which sources of anthocyanin-rich foods are most beneficial and the dosage of anthocyanin intake needed to induce acute and longer-term cognitive effects. Ultimately, this will allow statistical comparison between studies, and potentially lead to the translation of research into dietary messages about dietary anthocyanins for acute and longer-term cognitive benefits.

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