The acute and chronic effects of a freeze-dried wild blueberry powder (253 mg anthocyanins) on cognition, reading behaviour, and mood, in healthy seven to ten-year olds.

Submitted for the degree of Doctor of Philosophy

School of Psychology and Clinical Language Sciences

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Gabrielle May.

Abstract

Previous research in healthy young and older adults has reported significant improvements in several cognitive domains (executive function, processing speed, attention) following acute and chronic supplementation of anthocyanin-rich treatments. Most recently, research has started to examine whether similar anthocyanin-rich interventions can benefit children's cognition. To date, research has demonstrated significant improvements to episodic memory and executive functions following a single, one-off dose (acute) of a 30 g freeze-dried wild blueberry (WBB) beverage (253 mg anthocyanins) in healthy seven to ten-year-old children. This thesis aimed to extend the previous research in three ways. First, by investigating the effects of a daily dose of WBB consumed over four weeks (chronic) on children's cognition, in addition to the acute effects of WBB consumption. Second, by examining whether acute and chronic WBB supplementation exerted additional benefits to reading behaviour, and mood. Third, by examining whether acute WBB effects on performance differed as a result of time of day: morning versus afternoon testing.

The cognitive, reading behaviour, and mood, effects of a freeze-dried WBB powder beverage (253 mg anthocyanins) were investigated in four placebo-controlled, between-subjects experiments; treatments groups matched for age and gender. Acute effects of WBB supplementation were examined in three studies (Study 1: *N* = 54, Study 2: *N* = 61, Study 4: *N* = 40); the fourth explored both acute and chronic WBB effects (Study 3: *N* = 30). Consistent improvements to executive function (faster reaction times), as assessed by the Modified Attention Network Task, were demonstrated in response to both acute and chronic WBB relative to placebo. More specifically, these effects were demonstrated under conditions of high cognitive demand, for example, during faster executive function trials and during afternoon testing where children perform below their cognitive optimum. Acute WBB intake improved total word recall and delayed word recall on a list-wise word learning task (Rey's Auditory Verbal Learning Task) relative to placebo. Self-reported positive affect (Positive Affect and Negative Affect Scale for Children) improved following acute WBB consumption relative to placebo, whilst chronic WBB consumption maintained both positive affect and negative affect. In this instance, acute and chronic WBB supplementation did not benefit word reading (Test of Word Reading Efficiency) or learning to read words (orthographic learning task).

This research supports previous studies demonstrating WBB-related improvements on children's cognition and is the first to demonstrate acute WBB effects on mood, and chronic WBB effects on cognition and mood, in children. As reading is a cognitively demanding task, it was hypothesised that WBB-related effects might be further observed for reading; however this hypothesis was not supported. One explanation is that all children recruited had average to good reading ability. Therefore, this highlights whether WBB supplementation would benefit reading behaviour in this cohort of children. Instead, future research should consider whether WBB supplementation can improve reading behaviour in poor performers and those with a deficient habitual flavonoid intake.

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Table of Contents

Figures and Tables

Glossary of Abbreviations

AA	Arachidonic Acid
AD	Alzheimer's' Disease
ADHD	Attention-Deficit / Hyperactivity Disorder
ANT	Attention Network Task
ATP	Adenosine Triphosphate
AVLT	(Rey's) Auditory Verbal Learning Task
BAS	British Ability Scale
BB	Blueberry
BDNF	Brain-derived Neurotrophic Factor
BOLD	Blood Oxygen Level Dependent response
CBF	Cerebral Blood Flow
CGJ	Concord grape juice
CREB	Cyclic AMP response element binding
DHA	Docosahexaenoic Acid
EEG	Electroencephalography
EPA	Eicosapentaenoic Acid
ERK	Extracellular-signal-regulated Kinase
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric acid
GDS	Geriatric Depression Scale
GPC	Grapheme-phoneme Correspondences
GTPase	Guanosine Triphosphate

HDL-cholesterol	High Density Lipoprotein cholesterol
MANT	Modified Attention Network Task
MAO	Monoamine Oxidase
MCI	Mild Cognitive Impairment
MFT	Modified Flanker Task
MI	Myocardial Infarction
MVM	Multivitamin and mineral
MWM	Morris Water Maze
PANAS-C	Positive and Negative Affect Scale for Children
PMT	Picture Matching Task
POMS	Profile of Mood States
PUFA	Polyunsaturated Fatty Acid
RCT	Randomised Controlled Trial
RVIP	Rapid Visual Information Processing task
TMT	Trail-Making Task
TOWRE-2	Test of Word Reading Efficiency – 2nd Edition
TST	Task Switching Task
VAS	Visual Analogue Scale
WBB	Wild blueberry
WIAT-III	Wechsler Individual Achievement Test 3rd Edition

Chapter 1

An introduction to flavonoids, cognition, reading behaviour and mood.

The research detailed in this thesis investigates whether consumption of a flavonoid-rich wild blueberry (WBB) treatment elicits positive changes to the cognition, reading behaviour, and mood of children aged seven to ten years old. The research focuses on both acute and chronic intervention regimes to determine the nature of a single one-off dose of WBB (Study 1 in Chapter 2 & Study 2 in Chapter 3) and a chronic dose of WBB given daily over four weeks (Study 3 in Chapter 4) on these domains of interest. Further investigations were conducted to examine the influence of time of testing (morning vs. afternoon) on the impact of acute WBB supplementation on cognition, reading behaviour, and mood (Study 2 in Chapter 3), and to examine whether acute WBB intake could improve more specific aspects of reading: visual word learning (Study 4 in Chapter 5). First, Chapter 1 will provide a concise overview of: diet and cognition, reading, and mood, followed by a short summary of the empirical chapters that will explore the capability of dietary strategies to improve cognition, reading and mood.

1.1 Nutrition and cognition

Diet has been shown to impact cognition across the human lifespan (for a review see Power et al., 2019; Benton, 2010). Research has explored the phytochemical constituents of a variety of foods and beverages; particular interest has focused on the flavonoids, a class of compounds identified in recent years for their positive impact on cognitive performance. Although to date no research has examined the effect of flavonoid consumption on reading behaviour, research demonstrates a positive effect of flavonoid consumption on cognition (for review see Bell et al., 2016; Lamport et al., 2012; Macready et al., 2009). Evidence from both epidemiological (Letenneur et al., 2007) and intervention studies (Krikorian et al., 2010a; Krikorian et al., 2010b) shows that flavonoid consumption is linked to the delayed onset of ageing disorders, such as Alzheimer's disease, in the elderly (Boesfplug et al., 2017; McNamara et al., 2019). Further, reductions in cognitive impairments have been observed in healthy older adults with significant improvements to executive function and memory observed following acute flavonoid consumption (Bell et al., 2018; Whyte et al., 2018). In addition, research in young healthy adults demonstrates that flavonoids elicit positive benefits to higher level executive functions such as processing speed and sustained attention

(Watson et al., 2015; Dodd et al., 2019). Most recently, research has examined the effects of acute berry-flavonoid interventions on children's cognition, showing improvements to the memory and attention of school-aged children (eight to ten-year olds) after consumption (Whyte & Williams, 2015; Whyte et al., 2016). Collectively, the majority of berry-flavonoid research has observed significant improvements to the cognitive domains of episodic memory, executive functions, and working memory following treatment consumption (see Sections 1.1.1.5- 1.1.1.7 for a detailed review). These domains are not only relied upon for general cognitive functioning, but also for other skill sets. For example, all three domains are required for fluent and efficient reading ability. As reading is routinely taught to children in their primary-school years, and as berry-flavonoid interventions have been shown to improve aspects of cognition important for reading in children of the same age, berry-flavonoids may have the potential to improve reading behaviours in children.

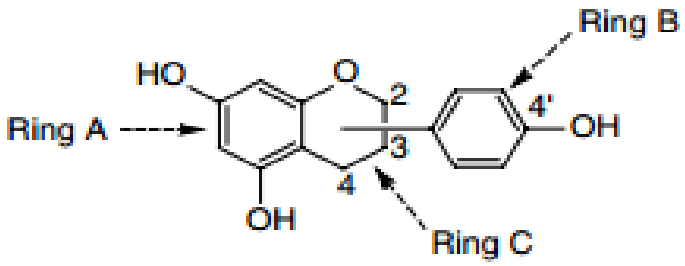
1.1.1 Flavonoids

In order to understand how flavonoids influence cognition, it is first important to understand how flavonoids impact the human body and their mechanisms of action once ingested.

1.1.1.1 What are flavonoids?

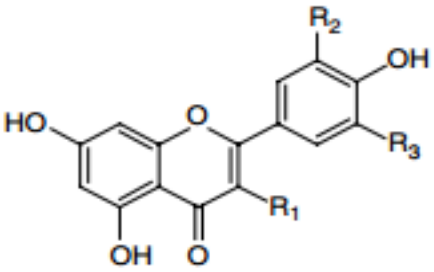
Flavonoids are a group of plant-derived phytochemicals readily available in the human diet: fruits, vegetables, tea, grains, wine, chocolate, and even bark extracts such as Gingko biloba. Flavonoids can be divided into six subclasses dependent on subtle differences in chemical structure: flavonols, flavones, isoflavones, flavanones, flavanols, and anthocyanins (Figure 1.1.1.1B; Rodriguez-Mateos et al., 2014). The general chemical structure of flavonoids consists of two aromatic carbon rings of benzopyran (A and B) which are bound together by three carbon atoms to form an oxygenated heterocycle of benzene (ring C). Differences in the level of C-ring oxidation, the hydroxylation of the general ring structure, and substitution of the C-3 ring position (Figure 1.1.1.1A) distinguishes the different subclasses.

A



B

Flavonol and Flavone



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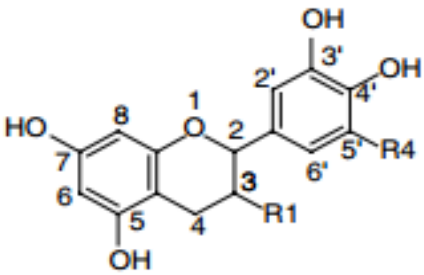
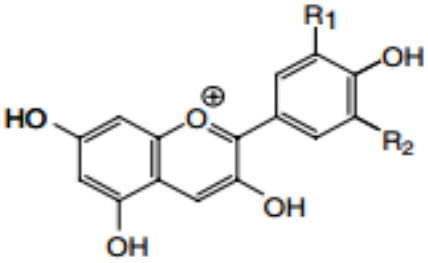
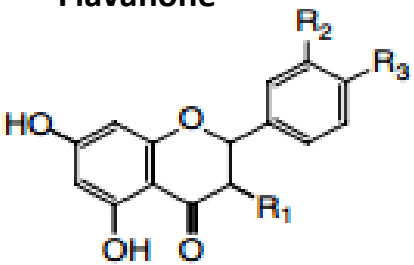


Anthocyanin



Flavanone



Isoflavone

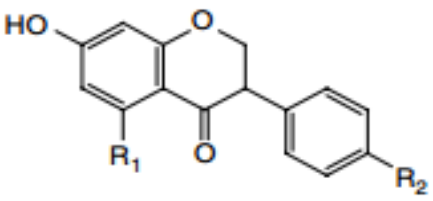


Figure 1.1.1.1: A) The general flavonoid structure (Manach & Donovan, 2004; adapted by Spencer, 2008), **B)** The chemical structures of the six flavonoid subclasses (adapted from Rodriguez-Mateos et al., 2014).

1.1.1.2 Flavonoid metabolism

Once ingested by the body, flavonoids first undergo phase I metabolism in the small intestine and liver which involves chemical reactions of oxidation and hydrolysis. Here, glycosides are deconjugated (detached from an ionisable group) or hydrolysed into aglycones allowing them to diffuse into the surrounding epithelial cells. When flavonoids undergo these chemical changes, molecules with greater polarity are produced which are soluble in water, allowing their diffusion and subsequent excretion. During phase I metabolism it is important to note that not all aglycones receive further metabolism. A small amount of flavonoid metabolites are excreted from the body or pass directly into the bloodstream. Flavonoids that are not excreted are subject to further metabolism with continued phase I and phase II metabolism occurring in the small intestine and, subsequently, in the liver.

During phase II metabolism, aglycones undergo sulphation, methylation, and glucuronidation through conjugation and detoxifying of the corresponding enzymes (sulphotransferases, catechol-*O*-methyltransferases, and UDP-glucuronosyltransferases). Here, the aglycones are de-glucosylated and metabolised further into glucuronides, sulphates and *O*-methylated derivatives before they pass into the bloodstream where potential action on target cells or excretion occurs. Metabolites that are not absorbed in the small intestine pass into the colon where colonic bacteria cause deconjugation, breaking metabolites down into simple phenolic acids (Manach & Donovan, 2004). These are then reabsorbed through the colon and transported back into the small intestine or the liver, via the hepatic vein, as a result of enterohepatic recycling. Here, phase I and phase II metabolism can recommence (Vauzour et al., 2008; Del Rio et al., 2010, Kalt et al., 2014; Thilakaranthna & Rupsasinghe, 2013; Figure 1.1.1.2).

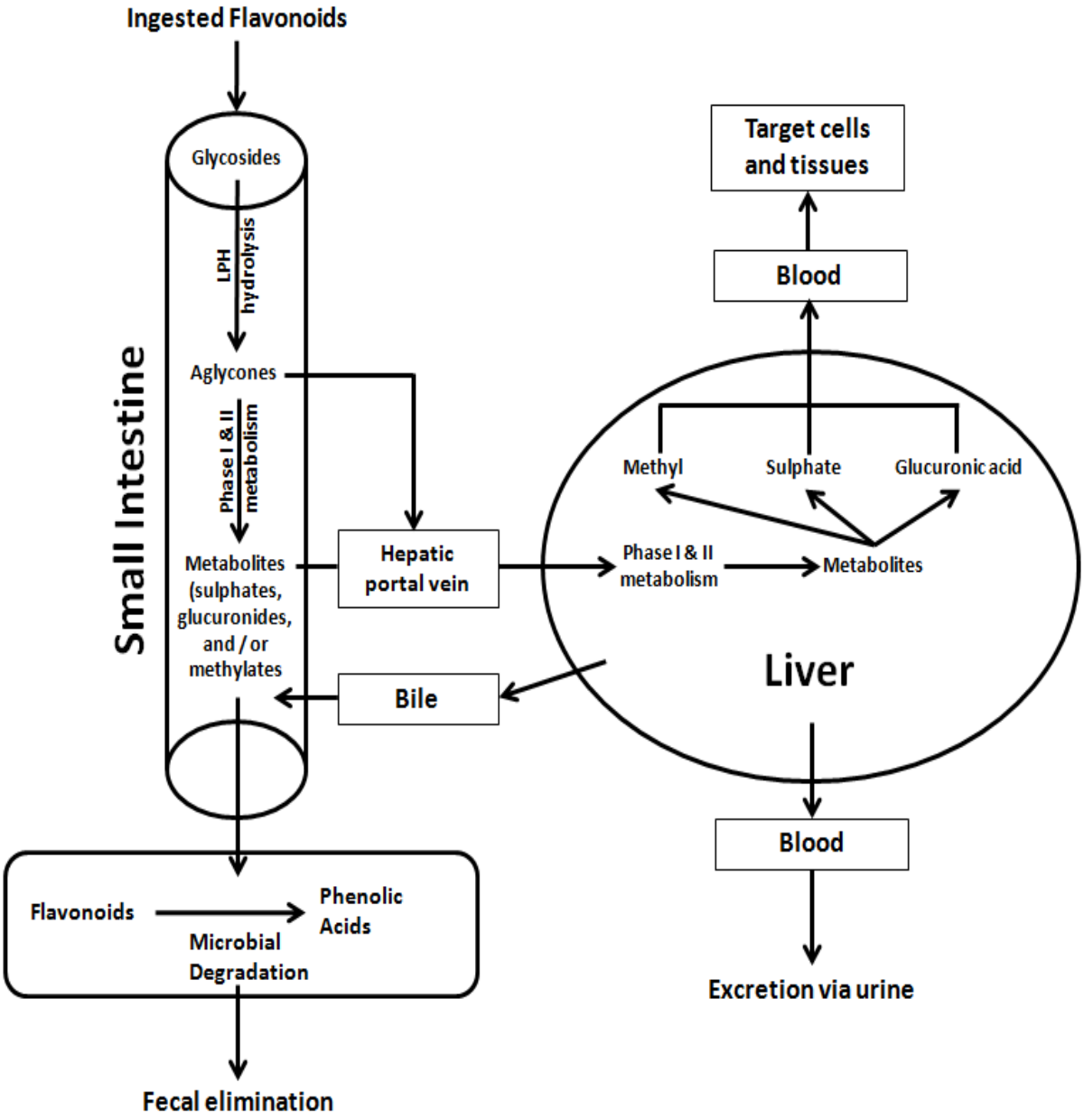


Figure 1.1.1.2: Schematic of human flavonoid metabolism adapted from Del Rio et al. (2010), Kalt et al. (2014), and Thilakarathna and Rupasinghe (2013). The figure indicates the location of phase I and phase II flavonoid metabolism, the primary metabolites, enterohepatic recycling, entry in the bloodstream, and eventual excretion/elimination.

1.1.1.3 Health benefits of berry flavonoids in humans: cardiovascular, diabetes, cancer

The sections below focus specifically on flavonoids found in berry fruits. There is an established literature detailing the health benefits of berry-flavonoids in humans, with respect to their importance for cardiovascular health, diabetes, cancer prevention, and neurological functioning.

Epidemiological studies highlight the cardiovascular benefits of diets rich in berry-flavonoids. Comprehensive longitudinal research by Cassidy et al. (2010; 2013) has observed an inverse association between the habitual intake of berry-flavonoids and the risk of myocardial infarction (MI) and hypertension. Specifically, intake of a minimum of two anthocyanin-rich fruits per week was associated with a decreased risk of MI compared to those with a lower weekly intake in women aged 25 to 42 years (Cassidy et al., 2013). Similarly, adults with a higher habitual intake of anthocyanins from blueberries and strawberries per day (15.2 mg/d) reduced their risk of hypertension compared to those with a lower habitual intake (12.5 mg/d; Cassidy et al., 2010).

Randomised controlled trials (RCTs) have further added to the above epidemiological research providing indications of cause-and-effect for berry-flavonoids on cardiovascular health. Decreases in systolic and diastolic blood pressures were observed following eight-week daily supplementation of a 160 g mixed berry treatment (515 mg/d anthocyanins) in participants with cardiovascular risk factors; further, inhibition of platelet function and increases in HDL-cholesterol concentrations were observed after treatment (Erlund et al., 2008). Further, Rodriguez-Mateos et al. (2013) observed that flow mediated dilation (FMD), a measure of endothelium-dependent vasodilation and a subsequent measure of cardiovascular disease, increased in relative proportion to the concentration of anthocyanin metabolites in the bloodstream of healthy young men following an acute 34 g blueberry treatment (310 mg anthocyanins).

Although the literature supporting the benefits of berry-flavonoids for type 2 diabetes is currently limited, evidence thus far is positive. Epidemiological work suggests an inverse association between habitual blueberry intake and the risk of type 2 diabetes (Wedick et al., 2012). Specifically, two servings of blueberries per week was associated with a lower risk of type 2 diabetes in healthy adults. Subsequent RCTs support the epidemiological research, indicating that berry-flavonoids improve insulin sensitivity and associated mechanisms of

type 2 diabetes. Postprandial research by Edirisinghe et al. (2011) demonstrated that an increase in plasma anthocyanins from a beverage containing 10 g strawberry powder (81.6 mg/10 g anthocyanins) significantly attenuated postprandial inflammatory responses, such as a reduction in insulin, when induced by a high carbohydrate, moderate fat meal. Similarly, when white bread, which typically induces high postprandial glucose and insulin responses, was consumed in parallel with a 150 g mixed berry treatment (no anthocyanin concentration provided), significant improvements were observed in the glycaemic profiles of healthy adolescent women. The results showed that berry-flavonoids reduced insulin levels needed for the maintenance of postprandial glucose metabolism (Törrönen et al., 2013).

Berry-flavonoids are also known for their chemopreventative properties. Although research mainly focuses on *in vivo* or *in vitro* studies, the limited studies conducted in humans are promising. Pan et al. (2015) examined the effect of daily chronic black raspberry treatment on patients with colon and rectal cancer. Three 20 mg daily doses of the black raspberry treatment (no anthocyanin concentration provided) were shown to positively modify tumour suppressor genes involved in proliferation, apoptosis and angiogenesis of cancerous cells. Similarly, Kresty et al. (2006) found that patients with Barrett's oesophagus, the abnormal growth of cells in the oesophagus and a precursor to cancer, who were supplemented with a daily dose of black raspberry (45 g/d males: 32 g/d females; no anthocyanin concentration given) over a six-month period showed a reduction in the urinary excretion of markers linked to cell death and damage: 8-epi-prostaglandin F2α (8-Iso-PGF2) and 8-hydroxy-2-deoxyguanosine (8-OHdG). Both markers have been previously linked to oxidative stress in preclinical (Chiou et al, 2003) and *in vivo* (Basu & Helmersson, 2005) studies, causing damage to cell DNA and lipid peroxidation.

1.1.1.4 Berry flavonoids and cognition

The sections below examine the effects of berry-flavonoids on cognition in preclinical rodent studies and in humans with focus on specific domains of interest: episodic memory, working memory, and executive function. Research studies supporting the role of berry-flavonoids on cognition are reported below under one domain only, dependent on the main significant finding reported by the authors. These cognitive domains have consistently demonstrated changes in performance following berry-flavonoid treatments. Further,

although working memory is classed as a subdivision of executive function, the preclinical literature highlights a number of robust improvements to working memory alone.

Episodic memory is a form of declarative or explicit memory, and refers to the processes that support the encoding, storage, and conscious recollection of events (Baddeley, 2001). Further, verbal episodic memory is the specific storage of phonological information. Working memory is a collection of storage systems such as the visio-spatial sketch pad, the central executive, the phonological loop, and the episodic buffer, which work simultaneously to keep information in mind whilst performing complex tasks such as reasoning, comprehension, and learning (Baddeley, 2010). Last, executive function details the group of cognitive processes that allow flexible responding to the environment and the engagement in deliberate, goal-directed thoughts and actions. Executive function skills include inhibition, shifting, and updating. It is these skills which form the basis of abilities such as problem solving and flexible thinking (Cragg & Gilmore, 2014).

1.1.1.4.1 Berry flavonoids and cognition in preclinical rodent models

Prior to human epidemiological studies and RCTs, pre-clinical rodent models have provided insight into the role of flavonoids on cognition with studies indicating that flavonoid-rich foods elicit improvements to several cognitive domains: memory acquisition, working memory, reversal learning, and memory retention/retrieval (for review see Rendeiro et al., 2012). A focus of the pre-clinical literature was the effect of berry-flavonoids on the spatial working memory of rodents (Table 1.1.1.4.1). Joseph et al. (1998) examined whether daily supplementation of either a strawberry extract, spinach extract, or vitamin-E extract (1-2% normal dietary intake; no anthocyanin concentrations provided) over an eight-month period could prevent the onset of age-related physiological changes and cognitive impairments in a sample of healthy rats (six to fifteen months olds). Spatial working memory performance assessed by the Morris Water Maze (MWM) showed a decrease in time spent finding the platform and distance swam to find the platform between trial one and trial two for all diets; the difference in spatial working memory performance following the spinach extract and vitamin-E extract was significantly different from the control diet. This cognitive finding was supported by significant positive changes to neurophysiological parameters. All diets showed significant improvements to dopamine release, Gamma-Aminobutyric acid (GABA) activity, synaptic calcium recovery, and Guanosine Triphosphate (GTPase) activity. In

line with the cognitive outcomes, these findings suggest that diets high in flavonoids can protect against age-related physiological changes and subsequent cognitive impairments.

Further research by Joseph et al. (1999) supports the previous findings as significant improvements in the same neurophysiological and cognitive outcomes were observed in older healthy rats (fifteen months of age). Following eight-month supplementation of a strawberry extract, spinach extract or blueberry extract (1-2% normal dietary intake; no anthocyanin concentrations provided), spatial working memory performance significantly improved between trials for all diets, with time spent finding the platform and distance swum to find the platform decreasing between trial one and trial two. Psychomotor performance on the rod walking and rotarod tasks assessing balance and coordination was further improved following supplementation of the blueberry extract only. Additionally, in line with the previous findings, significant improvements to the physiological measures assessed: calcium recovery, GTPase activity, dopamine release, were observed following all diets. Williams et al. (2008) focused specifically on the chronic effects of a blueberry supplemented diet (2% normal dietary intake; approximately 6.68 mg/d anthocyanins consumed) fed daily over a twelve-week period to older rats (eighteen months) on spatial working memory performance. Cognitive performance was compared to a control group of young rats (six months) and an equivalent group of older rats (eighteen months) fed a placebo diet. Those supplemented with the blueberry diet significantly improved their decision-making latency and choice accuracy for spatial working memory, as assessed via the cross maze apparatus, over the twelve week period compared to older rats fed the placebo diet. Additionally, the older rats who had received the blueberry intervention showed similar levels of hippocampal brain-derived neurotrophic factor (BDNF) and Cyclic AMP response element binding (CREB) at twelve weeks to the young rats. It was thought that these changes were due to increased activation in the ERK signalling pathways which potentially underlie the improvements seen for spatial working memory performance. The older rats fed the placebo diet showed detriments in BDNF and CREB level. Further support from Rendeiro et al. (2013) observed that chow supplemented with either pure anthocyanins (.179 mg), pure flavanols (.0741 mg), or blueberry powder with equivalent anthocyanin and flavanol levels (.179 mg anthocyanins, .0741 mg flavanols; 2% normal dietary intake) all significantly increased hippocampal BDNF levels in eighteen-month old rats; this in turn was correlated with improvements to spatial working memory performance. These findings highlight the

role of flavonoid treatments to maintain and improve cognitive functioning in healthy older animals, but they do not highlight the potential of flavonoids to reduce such deficits in those with induced neuropathology.

Shukitt-Hale et al. (2008) supplemented four-month old Fischer rats with either 2% blueberry (4.4 g/d; anthocyanin concentration not provided), .015% piroxicam (an anti-inflammatory drug), or a control diet for eight weeks before kainic acid (a neurotoxin known to induce neurodegeneration in important brain regions for learning and memory) was micro-infused into the hippocampus. Following the kainic acid treatment, spatial working memory performance in the MWM was improved in rats previously fed the blueberry supplemented diet for eight weeks, and showed no difference in latencies to find the platform on days two to four between the blueberry-kainic acid group and the control group. In addition, inflammatory responses in the hippocampus which occurred as a result of kainic acid micro-infusion were attenuated following blueberry treatment. Prior support comes from a study in which APP+PS-1 transgenic mice (genetically predisposed to Alzheimer's disease; AD) fed the same blueberry diet as employed by Shukitt-Hale et al. (2008) for eight months showed no deficits in Y-maze performance compared to controls fed a normal diet; the blueberry diet disrupted the effect of inflammatory markers in hippocampal neurons associated with AD pathology (Joseph et al., 2003). Wang et al. (2014b) further observed the amelioration of memory and learning deficits, and associated neurological pathologies (restoring mitochondrial membrane potential, ATP levels in hippocampal cell membranes, reduction of plaques and mitochondrial dysfunction in general) when 40 mg/kg quercetin (a common flavanol found within the human diet obtained from berry sources) was administered daily to APPswe/PS1dE9 transgenic mice (genetically predisposed to AD) for sixteen weeks. Overall, the findings support the possible attenuation of AD pathologies following consumption of berry flavonoids, protecting and enhancing cognitive functions in rodents.

Table 1.1.1.4.1: Summary of preclinical studies examining the relationship between anthocyanin consumption and cognitive performance.

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Joseph et al. (1998)	80 male Fischer 344 rats aged six months	One of four diets: 1) Corn starch control diet 2) Freeze-dried strawberry extract (9.5 gm/kg) added to corn starch 3) Freeze-dried spinach extract (6.4 gm/kg) added to corn starch 4) Vitamin E acetate (500 IU) added to corn starch	Eight-month, randomised, placebo controlled, between-subjects	Tested at eight months. Spatial working memory assessed by the Morris Water Maze (MWM) - twice daily (morning and afternoon) for four consecutive days (two trials per morning and afternoon session)	Spinach had a significantly shorter latency to find the platform compared to placebo. Spinach and vitamin E had a significantly shorter distance swum to find the platform compared to placebo
Joseph et al. (1999)	40 male Fischer 344 rats aged 19 months	One of four diets: 1) Corn starch control diet 2) Freeze-dried strawberry extract (9.5 gm/kg) added to corn starch 3) Freeze-dried spinach extract (6.4 gm/kg) added to corn starch 4) Freeze-dried blueberry extract (18.6 gm/kg)	Eight-week, randomised, placebo controlled, between-subjects	Tested at eight weeks. Spatial working memory assessed by the MWM (as reported by Joseph et al., 1998), psychomotor function assessed by: rod walking, wire suspension, plank walking, inclined screen, accelerating rotarod	Strawberry and blueberry spent had a significantly shorter latency to find the platform compared to placebo. All diets had a significantly shorter distance swum to find the platform compared to placebo. Strawberry and spinach had a significantly longer latency to fall for rod walking compared to placebo. Blueberry had a significantly longer latency to fall for accelerating rotarod compared to placebo

Table 1.1.1.4.1: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Rendeiro et al. (2013)	Four groups of male Wistar rats aged 18 months	One of four diets: 1) Control 2) 2% (w/w) blueberry powder (253.1 µg flavonoids/g) 3) Anthocyanin extract (179.0 µg 4) Flavanol extract (14.8 µg of pure epicatechin and catechin)	Six-week, randomised, placebo controlled, between-subjects	Tested weekly over six weeks. Spatial working memory assessed by the cross-maze apparatus (as reported by Williams et al., 2008)	All diets significantly improved choice accuracy at week four compared to control, with improvements maintained at weeks five and six
Shukitt-Hale et al. (2008)	70 male Fischer 344 rats aged four months	One of three diets: 1) Corn control 2) 2% (w/w) freeze-dried blueberry extract 3) .015% (w/w) piroxicam powder	Eight-week, randomised, placebo-controlled, between-subjects	Tested at eight weeks. Spatial working memory assessed by the MWM (as reported by Joseph et al., 1998)	Both diets had significantly shorter latency to find the platform on days two, three, and four compared to control diet
Wang et al. (2014b)	APPswe/PS1dE9 transgenic mice aged three months	One of four diets: 1) Control 2) 20 mg/kg quercetin 3) 40 mg/kg quercetin 4) 2 mg/kg Aricept	Sixteen-week, randomised, placebo-controlled, between-subjects	Tested ay sixteen weeks. Spatial working memory assessed by the MWM (as reported by Joseph et al., 1998)	Quercetin (40 mg/kg) had a significantly shorter latency to find the platform

1.1.1.4.2. Berry flavonoids and older adults with cognitive decline

The neuroprotective properties of berry-flavonoids observed in pre-clinical rodent models are further mirrored in healthy older adults and those with compromised neuropathology. Epidemiological research by Letenneur et al. (2007) found that healthy older adults (65 years and over) grouped into the two highest quartiles of flavonoid intake (13.6-17.69 mg/d; 17.7-36.94 mg/d) had significantly better cognitive performance (Mini-Mental State Examination) at baseline and at a ten-year follow-up compared to those with a low habitual intake (0-10.39 mg/d). Those with low intake exhibited a loss of 2.1 points in comparison to only a 1.2 point decrease for those with high intake over the ten year period. Similarly, a study by Devore et al. (2010) examined the habitual intake of dietary flavonoids in older women (70 years +) and their cognitive performance over a ten-year period. It was found that those who ate one to two portions of blueberries and strawberries per week showed a delay in their cognitive aging equivalent to 1.5-2.5 years compared to those who consumed one to two portions per month. Although epidemiological studies to date highlight an important association between flavonoids and protection against neurodegeneration and cognitive aging, RCTs are required to provide more controlled and rigorous examinations (Table 1.1.1.4.2).

1.1.1.4.2.1 Working memory

In general, human RCTs support the association between berry-flavonoid intake and cognitive improvements in those with neurodegeneration, and provide an indication of cause and effect. Berry-flavonoid intervention studies employing older adult samples with mild cognitive impairment (MCI), putting them at an increased risk of developing AD, indicate improvements to human neuropathology consistent with the above preclinical animal models where significant improvements to working memory were observed. However, in contrast to the preclinical literature, findings in older adults with MCI do not currently extend to working memory. Following sixteen-week daily supplementation of a 148 g/d blueberry treatment (14.53 mg/d anthocyanins), increased blood oxygen level-dependent (BOLD) activation was observed in the left pre-central gyrus, left middle frontal gyrus, and inferior parietal lobe when completing a working memory task whilst undergoing fMRI; however, no changes in working memory behavioural outcomes were seen (Boespflug et al., 2018). This is the first study to demonstrate heightened neural

responses to a blueberry treatment in older adults with cognitive deficits; although caution should be taken as no benefits to working memory performance were observed.

1.1.1.4.2.2. Episodic memory

Although the literature focusing on the effects of berry-flavonoids on working memory in cognitively impaired older adults is limited, there is support for an effect of berry-flavonoids on improving episodic memory in the same population. For example, a study which supplemented older adults (mean age: 78 years) exhibiting MCI with daily Concord grape juice (between 444-621 mg/d dependent on participant weight) or placebo over a twelve-week period observed significant improvements to verbal learning, as well as a near significant increase in verbal and spatial recall (Krikorian et al., 2010a). A follow-up study examined the effect of WBB juice in a similar subset of participants (Krikorian et al., 2010b). Older adults (mean age: 76 years) with MCI consumed a WBB treatment (between 444-621 mg/d dependent on participant weight) or placebo daily over a twelve-week period. A significant improvement to paired associate learning (Verbal Learning Test) was observed post-treatment compared to the placebo. One significant limitation of this study was the placebo used. Instead of being matched to the blueberry treatment, the placebo was matched to the Concord grape juice treatment used by the previous study (Krikorian et al., 2010a), but only for carbohydrate composition and energy load. Therefore, drink constituents such as sugars and vitamins were not evenly matched between the blueberry treatment and placebo, and potentially suggests that any constituent differences observed between this treatment and placebo could be contributing to the cognitive findings observed, not necessarily the blueberry anthocyanins.

Both above studies indicate that daily flavonoid supplementation over a chronic period ameliorates cognitive deficits associated with MCI, a precursor of AD. However, Krikorian et al.'s research is based on relatively small samples sizes (Krikorian et al., 2010a: *N* = 9; Krikorian et al., 2010b: *N* = 12) before participants were randomised to treatment groups. These small sample sizes, and subsequent lack of power, increase the risk of type I error when running statistical analyses and leaves a greater margin for individual differences to influence findings as were observed at baseline (Krikorian et al., 2010a). In particular, significant differences at baseline were observed for levels of depressive symptoms when assessed by the Geriatric Depression Scale (placebo = 7.8, grape juice = 3) suggesting that

greater depressive symptomology in the placebo condition may have resulted in poorer cognitive performance for the outcome measures completed compared to those receiving grape juice. Further, differences in age were observed (placebo = 80 years, grape juice = 75 years), allowing the treatment group an unfair advantage in terms of cognitive performance at this sensitive period in cognition over the human lifespan. This suggests that any differences observed between treatments could have been influenced by these individual differences seen at baseline. This leads to difficulties with establishing the true effects of flavonoids on the episodic memory of those with cognitive decline and AD or MCI pathology. In addition, both studies utilised treatments which varied according to participant body weight, meaning the treatment dose wasn't consistent across participants. The cognitive measures used above were limited and only focused on those sensitive to decline with the onset of ageing disorders, therefore it cannot be concluded from these studies that berry-flavonoids slow cognitive decline in other areas of cognitive function aside from word recall assessed here by Krikorian et al.

More recently, Kent et al. (2017) supplemented older adults (aged 70 years or above) with mild-to-moderate dementia with a daily 200ml anthocyanin-rich cherry juice (138 mg anthocyanins) over a twelve-week period. Cognition was assessed on a range of measures including: word recall and recognition, working memory, executive function, and short-term memory. Physiological measures were further assessed alongside cognition. After twelve weeks, chronic cherry juice supplementation significantly improved cognitive performance on the majority of measures. Total word recall and delayed word recall exhibited greater recall of words after treatment compared to placebo. Category verbal fluency performance was also seen to improve after twelve weeks treatment where the cherry juice intake exhibited greater performance compared to placebo. Further, a decrease in systolic blood pressure and a trend towards a reduction in diastolic blood pressure was observed twelve weeks after cherry juice consumption. Together these findings suggest that chronic supplementation of a flavonoid-rich cherry juice can significantly improve word recall and recognition, and executive function, and other health associated effects.

Further, Whyte et al. (2018) examined the effect of chronic WBB interventions over six months on the episodic memory, working memory, and executive functions of older adults with subjective memory complaints. A total of 115 healthy participants aged 65 to 80 years were randomly allocated to receive either a 500 mg WBB powder (1.35 mg anthocyanins), a

1000 mg WBB powder (2.7 mg anthocyanins), a 111 mg WBB extract (7 mg anthocyanins), or a placebo on a daily basis. Cognitive function was assessed via test battery: verbal episodic memory (AVLT), visual episodic memory (object recognition), spatial episodic memory (Corsi Blocks task), working memory (serial subtractions, Sternberg memory scanning), executive function (MANT, Stroop task), at baseline, three months, six months, alongside measures of vascular function. For verbal episodic memory, significantly greater performance was observed following consumption of the WBB extract in comparison to the placebo at three months. Similarly for spatial episodic memory, a trend was observed for those receiving the WBB extract with greater performance at three months in comparison to the placebo. These cognitive findings were in line with a decrease in systolic blood pressure at three months following consumption of the WBB extract, indicating a possible mechanism by which flavonoids exert their cognitive benefits. No other cognitive benefits were observed. Although no effects on cognition following consumption of either WBB powder were seen, this does not rule out the potential for cognitive improvements post-WBB powder consumption as had been observed previously. In this case, the anthocyanin concentrations in the powders, and in the extract, were significantly lower than those used by other WBB intervention studies; for example the acute freeze-dried WBB powder used by Whyte et al. (2016; 2017) contained 253 mg anthocyanins. Although significant improvements were observed following intake of the WBB extract, the lower anthocyanin concentrations may not be sufficient enough to observe other significant cognitive effects. In this instance, the significant findings, following consumption of the extract, may be attributed to its greater concentration of anthocyanins.

Although a significant improvement for verbal episodic memory was observed following intake of the WBB extract, with an additional trend for improved spatial episodic memory again following consumption of the extract, no other cognitive effects where observed throughout the study. Further, the improvement to episodic memory was only observed for one measure of word recall and recognition. It is important to consider the effect of type I error when conducting multiple comparisons, where an increased number of comparisons can lead to an increased chance of obtaining a false positive result. Although Whyte et al. (2018) applied Bonferroni corrections to combat the effect of type I error, this is still something to consider when reviewing the data. Next, the significant effects were only observed at three months, none were apparent at six months. Whyte et al. (2018) noted

that this could be due to practise effects between the test sessions. Here, participants may have adapted their strategy for completing the cognitive tasks at six months compared to three months, thus reducing their sensitivity to the treatments. In addition, the lack of treatment effects at six months could be due to degradation of the active components of WBB over the test period as Whyte et al. (2018) stated the contents of the treatments were not reanalysed at the end of the six-month period. Whyte et al. (2018) further suggested the lack of treatment effects could be a result of increased tolerance to the treatments. This is in contrast to other studies examining the chronic effects of berry-flavonoids on episodic memory where improvements have been observed over a period of several weeks and months.

Last, a study by McNamara et al. (2019) examined the effect of a 25 g BB treatment (269 mg/d anthocyanins) consumed daily over a 24-month period on the cognition of older adults suffering with mild, self-perceived cognitive decline without a clinical diagnosis. In comparison to a fish oil treatment or a combined BB/fish oil treatment, the BB treatment alone was shown to reduce cognitive symptoms noted initially at baseline through use of the Dysexecutive Questionnaire (Burgess et al., 1998; Chan, 2001); used to characterise self-assessed changes in the cognitive effectiveness of everyday activities with particular sensitivity to working memory and executive functions. These significant changes in Dysexecutive Questionnaire performance were seen at 24 weeks and 48 weeks throughout the chronic treatment period. Additionally, consumption of the BB treatment was found to significantly improve discrimination in recognition memory on the Hopkins Verbal Learning Task used as a measure of new learning, long-term memory, cumulative learning, delayed recall, and item discrimination. This study highlights that berry-flavonoid treatments can indeed improve episodic memory in older adults with mild, self-reported cognitive decline. When considered alongside Whyte et al. (2018) and Kent et al. (2017), these studies collectively demonstrate that berry-flavonoid supplementation has a beneficial effect on cognition in older adults with neurodegeneration when appropriately powered studies with balanced treatment group *n* numbers and matched treatments/placebos are employed.

Table 1.1.1.4.2: Summary of studies examining the relationship between anthocyanin consumption and cognitive performance in older adults with cognitive decline.

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Boespflug et al. (2018)	Nine female, seven male mean age 77.95 years. All met the clinical criteria for MCI with level of cognitive decline determined by the modified Clinical Dementia Rating	148 g/d freeze-dried whole fruit blueberry powder (14.53 mg/d anthocyanins) divided over two daily servings. Placebo matched for appearance, sugars, citric acid, carbohydrates, fats, protein	Sixteen-week, randomised, double-blind, placebo-controlled, between-subjects. n = eight blueberry, n = eight placebo	Tested at baseline and sixteen weeks. Working memory – n-back task, whilst undergoing fMRI	No significant improvements to working memory. Significant increase in BOLD responses during n-back task in: left pre-central gyrus, left middle frontal gyrus, left inferior parietal lobe
Krikorian et al. (2010a)	Four female, eight male mean age 78 years. All scored mild impairment on the Clinical Dementia Rating scale	Concord Grape Juice (Welch's). 6–9 ml per kg divided over three servings a day. Placebo matched for appearance, taste, carbohydrate, and energy	Twelve-week, randomised, double-blind, placebo controlled, parallel groups. n = five for grape juice, n = seven for placebo	Tested at baseline and twelve weeks. California Verbal Learning Test (CVLT), Spatial Paired Associated Learning Test	Grape juice had significantly greater memory acquisition at twelve weeks compared to control. No effects for delayed verbal memory or spatial memory

Table 1.1.1.4.2: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Kent et al. (2017)	49 older adults mean age 79.8 years. All recruited from a geriatric dementia clinic with diagnosis of mild-to-moderate dementia	200ml/d cherry juice (69 mg per 100 g anthocyanins). Placebo was 220 ml/d apple juice.	Twelve-week, randomised, double-blind, placebo-controlled, between subjects. n = 24 cherry juice, n = 25 placebo	Tested at six weeks and twelve weeks. AVLT, self-ordered pointing task, Boston naming test, TMT, digit span backwards task, category and letter verbal fluency.	Cherry juice had significantly greater category verbal fluency, AVLT delayed recall at both six weeks and twelve weeks compared to control
Whyte et al. (2018)	75 female, 47 male mean age 70.8 years. All scored above 24 on the Mini-Mental State Examination (MMSE)	One of four diets (ThinkBlue™, Naturex Inc): 1) Sugar and vitamin-C matched placebo 2) 500 mg WBB powder 3) 1000 mg WBB powder 4) 111 mg WBB extract	Six-month, randomised, double-blind, placebo-controlled, between-subjects	Tested at baseline, three months and six months. AVLT, picture recognition task, corsi block task, Stroop task, MANT, serial 3s and 7s, Sternberg task	WBB extract had significantly greater delayed word recognition and showed a trend towards greater corsi blocks correct sequence recall at three months compared to control and the powders

Table 1.1.1.4.2: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
McNamara et al. (2019)	41 female, 35 male mean age 68 years. All had self-perceived MCI	One of three diets: 1) Corn oil control 2) Fish oil capsule (400 mg/d EPA and 200/d mg DHA) 3) Freeze-dried whole fruit WBB powder (269 mg anthocyanins) 4) WBB + fish oil	Six-month, randomised, double-blind, placebo-controlled, between subjects. n = 17 fish oil, n = 19 WBB, n = 20 WBB + fish oil, n = 20 placebo	Tested at baseline, six months, twelve months. TMT part A and B, controlled oral word production, Hopkins verbal learning test	WBB had significantly greater recognition memory discrimination on the Hopkins verbal learning test at six months compared to control

1.1.1.4.3 Berry flavonoids and cognition in healthy adults

Evidence from preclinical studies demonstrates that berry-flavonoids not only enhance cognition in rodents with neurodegeneration, but also in healthy rodents (Joseph et al., 1998; 1999; Williams et al., 2008). This finding is further supported by human RCTs recruiting from both healthy young and older adult populations (Table 1.1.1.4.3).

1.1.1.4.3.1 Working memory

When examining the chronic effects of a daily 30 ml WBB treatment (387 mg/d anthocyanins) over a sixteen-week period, Bowtell et al. (2017) observed a significant improvement to working memory performance on a two-back task. In addition, participants also completed the Stroop task assessing executive function at sixteen weeks post WBB treatment whilst undergoing fMRI. Although no cognitive improvement was seen on Stroop task performance, increases to CREB and ERK (extracellular-signal-regulated kinase) signalling pathways and increased activation of cerebral blood flow (CBF) in task-related brain regions (anterior cingulate, precuneus, middle frontal gyrus, angular gyrus) were observed. This study supports evidence from preclinical rodent models and dose-response studies in humans which demonstrate increased CBF and enhanced neurogenesis and neuronal signalling within brain regions associated with cognitive performance following WBB supplementation (Section 1.1.1.4.1). Although the study fails to find cause-and-effect between the potential underlying mechanisms of action highlighted by fMRI and the Stroop task, this does not rule out the potential for these, and other similar mechanisms of action, to be driving improvements to other areas of cognition, such as working memory. Indeed, other studies employing the Stroop task to assess the effects of berry-flavonoid supplementation on working memory have also failed to see improvements for Stroop performance. These findings suggest the Stroop task may not be sensitive to the effects of berry-flavonoid supplementation on working memory performance. This highlights the need for future studies to consider using other working memory tasks such as the n-back task to observe potential working memory improvements following berry-flavonoid supplementation alongside the known neurological changes seen when undergoing fMRI.

In support of the positive benefits of berry flavonoids on working memory, a further study by Nilsson et al. (2017) examined the effect of a chronic 350 g mixed berry treatment (approximately 414 mg/d anthocyanins) administered three times a day over five weeks on

the cognition of healthy 50 to 70-year olds. Performance on a verbal working memory test significantly improved following the mixed berry treatment compared to placebo, with a 5% increase in performance observed at the five-week test session. Although no physiological measures were taken alongside cognitive testing for the present study, this finding supports that flavonoid-rich berry interventions can positively affect working memory performance following chronic supplementation. The two studies detailed here highlight the need for further research to conduct simultaneous neuroimaging and cognitive testing on the cognitive domains known to be susceptible to berry-flavonoid treatments.

The studies detailed above highlight that chronic berry-flavonoid treatments can improve working memory in heathy adult populations. These findings provide support for initial preclinical studies, as well as Bowtell et al. (2017) suggesting potential underlying mechanisms of action; for example, the effect of berry-flavonoids on increasing neuronal signalling pathways within task-related brain regions could be a potential reason for the observed cognitive changes.

1.1.1.4.3.2 Episodic memory

Dodd et al. (2019) conducted a cross-over study examining the effects of a 30.1 g acute flavonoid-rich BB treatment (579 mg anthocyanins) on the cognition of older adults, as well as cardiovascular markers and BDNF levels, at two and five hours post-treatment. Although originally assessing global cognitive function which was not affected by the BB treatment, individual analysis of immediate word recognition revealed a significant improvement after the BB treatment compared to placebo, with a significant difference in the number of words recalled between baseline and two hours after treatment. Although not significant in this case, BDNF plasma concentrations were seen to decline following the placebo whereas this decline was attenuated following consumption of the BB treatment. BB intake also attenuated the increase in systolic blood pressure observed after the placebo. The inclusion of these physiological measures, as with the research by Bowtell et al. (2017), starts to highlight the potential mechanisms of action of berry-flavonoid treatments which may be causing these positive cognitive changes. Thus far, the research in healthy adults suggests multiple mechanisms of action by which berry-flavonoids can induce these cognitive improvements to episodic memory.

An acute dose-response study by Bell et al. (2018) compared the effects of three haskap berry extracts (100 mg, 200 mg, 400 mg anthocyanins) on cognition, mood, and blood pressure in healthy older adults (aged 62-81 years). Supplementation of both the 200 mg and 400 mg doses significantly improved word recognition scores on an episodic memory task; Rey's Auditory Verbal Learning Task, one and a half hours after treatment administration. Here, intake of the 200 mg dose had a significantly greater number of words recognised compared to the 100 mg treatment and the placebo whilst intake of the 400 mg dose induced significantly greater performance compared to placebo only. Delayed word recall was also seen to improve following consumption of the 200 mg haskap berry extract after a 25-minute delay. Diastolic blood pressure was significantly lowered following consumption of the 400 mg dose. In this instance, there was no effect of haskap berry supplementation on working memory, executive function, or mood. The positive effect for episodic memory is consistent with the above literature (Section 1.1.1.4.3.2), demonstrating that episodic memory in older adults has been shown to be susceptible to change in several different berry-flavonoid RCTs.

When considering the effect of berry-flavonoids from other dietary sources on episodic memory, Lamport et al. (2016) observed significant improvements to Visual Spatial Learning Task (VSLT) immediate recall and Visual Verbal Learning Task (VVLT) immediate recall in healthy, middle-aged working mothers (aged 40-50 years) following twelve-week chronic supplementation of 355 ml/d Concord grape juice (CGJ; 167 mg/d anthocyanins). The cross-over study observed significantly greater immediate recall for both VSLT and VVLT tasks following consumption of the CGJ compared to placebo, but only when the CGJ was consumed first (study arm one) These findings remained apparent when participants next consumed the placebo (study arm two) suggesting that the positive changes in episodic memory following CGJ positive were maintained beyond the treatment's consumption period. However, this further highlights the importance of an appropriate length of wash-out period in cross-over design studies. In this case, the long-lasting effects of CGJ on episodic memory may have interfered with the effect of placebo on performance when consumed after the treatment; note this was not the case when placebo was consumed before treatment. Overall, these findings demonstrate that episodic memory improvements following chronic CGJ are maintained after ceasing CGJ consumption.

1.1.1.4.3.3 Executive Function

Recent research by Whyte et al. (2019) examined the effect of a 400 ml acute mixed berry treatment containing strawberries, blueberries, and raspberries (254.65 mg anthocyanins) on the executive function of young healthy adults aged 20 to 30 years. Mood was further examined (Section 1.1.1.5). Performance on two executive function tasks: Modified Attention Network task (MANT) and Task Switching task (TST), was assessed over a six-hour period to simulate the effects of berry-flavonoids over a working day. In general, MANT and TST accuracy performance declined over the six-hour period following placebo supplementation. In contrast, MANT accuracy significantly improved six hours after treatment supplementation compared to the placebo for the more cognitively demanding trials (incongruent trials). MANT reaction times were significantly faster at two hours and four hours following intake of the mixed berry treatment compared to placebo for these more cognitively demanding trials. Further, this finding was parallel to significantly faster TST reaction times at six hours compared to two hours indicating improvements to reaction times at the point when participants would be expected to experience cognitive fatigue. No such benefit was observed for placebo. These findings demonstrate the ability of a one-off flavonoid-rich treatment to maintain executive function performance over a six-hour period, as well as overcoming cognitive fatigue by improving executive function performance for more cognitively demanding trials.

Miller et al. (2019) observed similar improvements to executive function following daily consumption of a 24 g/d BB treatment (19.2 mg/g anthocyanins) over a 90-day period. Healthy older adults aged 60 to 75 years completed cognitive measures of: Attention Network Task (ANT), TST, trail-making test parts A and B (TMT-A/B; part A measures motor skills and visual search whilst part B measures cognitive flexibility), verbal learning, digit span, and a virtual Morris Water Maze (MWM) at day 45 and day 90. Mood was further assessed (Section 1.1.1.5). A significant improvement in TST accuracy was observed following 90 days of BB supplementation with a reduced number of switch errors observed compared to the placebo. Further, fewer repetition errors were observed at day 90 on the verbal learning task after BB intake compared to the placebo. Although this study reports significantly improved executive function accuracy on the TST, providing support for the findings of Whyte et al. (2019), no further BB effects were observed for the other executive function measures. The researchers suggest that the TST provides a more sensitive measure

of executive function compared to the TMT Part B as it is more attuned to changes in mental flexibility; as a result of the repetitive nature of the task procedure compared to the TMT. However, this does not explain the lack of BB effects on the ANT, a further robust measure of executive function as observed by Whyte et al.'s research (Whyte et al., 2016; 2017; 2018; 2019).

An acute cross-over study investigated the effect of two blackcurrant interventions (extract: 552.33 mg anthocyanins, or juice: 571.06 mg anthocyanins) versus a placebo on the cognitive performance of healthy 18 to 35-year olds (Watson et al., 2015). Performance was assessed over seven repetitions of a cognitive test battery including: a digit vigilance task, Stroop task, and rapid visual information task (RVIP), occurring repeatedly between 70 to 150 minutes post-treatment. The two blackcurrant treatments varied in their effect on performance; intake of the juice elicited significant improvements to digit vigilance reaction time compared to the extract or placebo, whilst intake of the extract significantly increased accuracy on the RVIP compared to the juice and placebo. This increase in RVIP accuracy remained throughout the cognitive battery repetitions (repetitions one, four, seven respectively), whereas an attenuation in digit vigilance reaction time was observed across all seven repetitions of the test battery. Additional differences were observed between the two treatments in terms of the physiological measures assessed. The juice treatment, being 20% higher in anthocyanins, resulted in the inhibition of blood platelet monoamine oxidase-B activity (involved in mood regulation) at two and a half hours post-intervention. This change was not prevalent after consumption of the extract. This physiological measure provides a potential explanation for why initial acute improvements were observed for digit vigilance following consumption of the blackcurrant juice, and the maintenance of digit vigilance performance over the repeatedly administered test battery. The behavioural findings support that berry-flavonoid rich treatments positively impact attention-based tasks.

A further study by Haskell-Ramsay et al. (2018) examined the effect of an acute 200 ml purple grape juice (138.8 mg anthocyanins) on the executive function and memory of a small sample (*N* = 20) of 18 to 35-year olds. Significantly faster reaction times on a composite attention measure were observed 20 minutes after consumption of the purple grape treatment compared to placebo, however no other significant cognitive outcomes were observed. Despite this significant improvement to executive function reaction time, a limitation of this study concerns the other cognitive domains of interest assessed. Although

a number of other cognitive domains were assessed in the cognitive test battery, according to the authors they were grouped into two broad subgroups: memory or attention. In terms of the memory subgroup, this seems a rather broad category to group cognitive domains such as: immediate and delayed word recall, numeric working memory, word recognition, picture recognition. This means that the subgroup of memory was assessing a mix of episodic memory and working memory domains which are known to respond differently to the effects of berry-flavonoids. In this instance, there was no mention of the tasks being analysed individually and indicates the potential for a masking of results for these individual domains; here, a negative outcome could be masking a positive outcome or vice versa. Further, this improvement in executive function reaction time could be a result of practise effects due to the unrealistic expectation of observing berry-flavonoid effects in such a short time period after ingestion. The acute effects of the purple grape juice treatment on cognition were assessed 20 minutes after treatment administration. Indeed, although research demonstrates anthocyanin excretion (up to 5%) as early as 24.5 minutes after strawberry consumption (Felgines et al., 2003), this does not necessarily mean that these anthocyanins underwent sufficient metabolism within this space of time. Further, Rodriguez-Mateos et al. (2013) observed biphasic peaks of anthocyanins within the bloodstream at one to two and six hours after consumption of a BB treatment (766 mg total polyphenols), alongside secondary improvements to cardiovascular measures such as flow-mediated dilation. Therefore, the lack of other cognitive findings by Haskell-Ramsay et al. (2018) suggests that this improvement in executive function reaction time is likely due to practise.

In general, the effects of berry-flavonoids on executive function in healthy adults span across a range of executive functions for both accuracy and reaction time outcome measures. Although there is an inconsistency in the domains of executive function improved as all studies report significant improvements to different executive functions, acute and chronic berry-flavonoid treatments demonstrate positive benefits particularly to response interference, sustained attention, mental flexibility, and working memory.

Table 1.1.1.4.3: Summary of studies examining the relationship between anthocyanin consumption and cognitive performance in healthy older and young adults.

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Bowtell et al. (2017)	13 female, 13 male mean age 68 years	30 ml blueberry concentrate from (BlueberryActive; 387 mg anthocyanins). Placebo was a synthetic blackcurrant and apple cordial (Robinsons cordial) matched for sugar	Twelve-week, randomised, double-blind, placebo-controlled, between subjects. n = twelve blueberry, n = fourteen placebo.	Tested baseline and twelve weeks. Detection task, Groton maze timed chase test, Groton maze learning test, identification task, international shopping list task, n-back memory tasks. Stroop test (whilst undergoing fMRI)	No effects of treatment during Stroop but blueberry significantly improved 2-back working memory performance. Blueberry had significant increases in BOLD responses in task related brain regions: Brodman areas 4, 6, 10, 21, 40, 44, 45, precuneus, anterior cingulate, insula, thalamus. Blueberry significantly increased resting-state perfusion of gray matter in parietal and occipital lobes
Nilsson et al. (2017)	30 female, 10 male mean age 63 years	600 ml mixed berry beverage: 150 g blueberries, 50 g blackcurrant, 50 g elderberry, 50 g lingonberries, 50 g strawberry, 100 g tomatoes (Orkla Foods Sverige AB; 414.2 mg/l). Placebo matched for sugars, pH, volume	Five-week, randomised, placebo-controlled, cross-over with 35 day wash out	Tested at baseline, two weeks and four weeks, at 30 mins, 90 mins and 150 mins on each test day after a standardised breakfast. Verbal working memory test, selective attention test	Mixed berry beverage had significantly greater working memory at the 30 mins test session at four weeks compared to placebo

Table 1.1.1.4.3: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Lamport et al. (2016)	25 female, mean age 42.8 years. All mothers of pre-teen children	355 ml Concord grape juice (Welch Foods; 167 mg/d anthocyanins). Placebo matched for energy, taste, appearance	Twelve-week, randomised, placebo-controlled, cross-over with a four week wash out	Tested at baseline, six weeks and twelve weeks. Visual verbal learning test immediate recall and delayed recall, visual spatial learning test immediate recall and delayed recall, RVIP, Grooved Pegboard, Tower of Hanoi	Concord grape juice had significantly greater visual verbal learning immediate recall if consumed before placebo and maintained performance into the placebo arm of the study. Concord grape juice had significantly greater visual spatial immediate recall compared to placebo
Dodd et al. (2019)	10 female, eight male mean age 68.7 years	30.1 g freeze-dried whole fruit blueberry powder (579 mg anthocyanins). Placebo was matched for sugars, vitamin-C, taste	Acute, randomised, placebo-controlled, cross-over	Tested at baseline, two hours and five hours. Global cognitive function: Go-NoGo, Stroop, Digit Switch, CPT, Digit Symbol Substitution Test, Random Word Generation, Three-Word Sets Task, n-back, letter memory, Location Task, immediate and delayed recall and recognition	Placebo had significantly worse global cognitive function at two hours compared to five hours and blueberry. Blueberry had significantly greater immediate word recognition at two hours compared to placebo.

Table 1.1.1.4.3: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Bell et al. (2018)	11 female, 9 male mean age 70.5 years	Three Haskap berry extracts: 100 mg, 200 mg, 400 mg anthocyanins. Placebo was matched for sugars	Acute, randomised, double-blind, placebo-controlled, cross-over with one week wash out	Tested at baseline and 90 mins. AVLT, Serial 3s and 7s, ANT	200 mg had significantly lower AVLT proactive interference as well as significantly greater Serial 3s correct responses compared to 400 mg and placebo. 400 mg had significantly greater AVLT delayed recognition compared to placebo
Whyte et al. (2019)	40 adults mean age 22.8 years	400 ml mixed berry smoothie: 75 g strawberry, 75 g blueberry, 75 g blackberry, 75 g raspberry (254.65 mg anthocyanins). Placebo was matched for sugars and vitamin-C	Acute, randomised, single-blind, placebo-controlled, between-subjects. n = 20 mixed berry, n = 20 placebo	Tested at baseline, two hours, four hours, and six hours. MANT, TST	Mixed berry had significantly greater MANT accuracy at six hours compared to placebo, for the more cognitively demanding incongruent trials. Mixed berry had significantly faster MANT responses at all time points compared to placebo, for the more cognitively demanding incongruent trials and for the low/medium load trials. Mixed berry had significantly faster TST responses at all time points

Table 1.1.1.4.3: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Miller et al. (2019)	24 female, 13 male	24 g/d freeze-dried blueberry (348 mg anthocyanins). Placebo matched for energy and flavour	90-day, randomised, double-blind, placebo-controlled, between-subjects.	Tested at baseline, 45 days and 90 days. CVLT, TST, TST, TMT, digit span, and a virtual MWM	Blueberry had significantly fewer repetition errors on the CVLT as well as a reduced switch cost on the TST at 90 days compared to placebo
Watson et al. (2015)	36 adults	Blackcurrant juice extract (Delcyantm; 525 mg anthocyanins), 142 ml cold pressed blackcurrant juice (Blackadder; 525 mg anthocyanins). Placebo was matched for sugars, sweetener, flavouring	Acute, randomised, double-blind, placebo-controlled, cross-over with a seven day wash out	Tested at baseline and 65 mins. Digit vigilance task, Stroop test, RVIP, logical reasoning test. The cognitive test battery was repeated seven times to induce cognitive fatigue	Blackcurrant juice had significantly faster digit vigilance responses at repetitions 1, 4, 7. Blackcurrant extract attenuated the reduction in RVIP accuracy compared to control, irrespective of repetition.

Table 1.1.1.4.3: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Haskell-Ramsay et al. (2018)	13 female, 7 male mean age 21.05 years	230 ml purple grape juice (Welch™) and blackcurrant flavour cordial (Schweppes™; 138.3 mg/l). Placebo made from 200 ml white grape (Welch™) and 10 ml blackcurrant flavour cordial (Schweppes™; 1.04 mg/l), matched for sugars	Acute, randomised, placebo-controlled, cross over with a seven day wash out	Tested at baseline and 20 mins. Computerised Mental Performance Assessment System (COMPASS): word presentation, immediate word recall, picture presentation, simple reaction time, digit vigilance, choice reaction time, numeric working memory, delayed word recall and recognition, delayed picture recognition. Composite scores calculated for: memory accuracy, memory reaction time, attention accuracy, attention reaction time	Purple grape juice had significantly faster attention reaction times compared to placebo

1.1.1.4.4 Berry flavonoids and cognition in children

Most recently, research has started to examine the effect of berry-flavonoids on cognition in children, specifically those aged seven to ten (Table 1.1.1.4.4). At this age, children experience a spurt in frontal lobe growth. This is thought to coincide with a critical period of development in their ability to competently perform tasks assessing cognitive domains such as executive function and memory (Cartwright, 2009; Davidson et al., 2006). Although cognitive domains such as goal setting and attentional control can be observed in children as young as three to five years old (Welsh et al., 1999; Espy, 1997), these executive functions are not adequately formed until seven to ten years old (Anderson et al., 2001) and are matured by the age of twelve (Anderson, 2002). Successful development of executive function domains between the ages of seven to ten years helps pave the way for educational success during adolescence as well as academic and occupational success in adulthood. However, poor development of these skills at this age can lead to executive dysfunction in children and consequently lead to poor impulse control, difficulties in monitoring and regulating performance, planning and organisational problems, and poor reasoning ability. Executive dysfunction can have a further negative affect on emotional responses and behaviour (Gioia et al., 2000) which can contribute to learning and behavioural disorders such as Attention Deficit Disorder (Sjöwall et al., 2013).

As cognitive domains such as episodic memory and executive function are known to benefit from berry-flavonoid supplementation in adults (Section 1.1.1.3), this highlights the potential of berry-flavonoid supplementation to benefit children's cognition. In particular, berry-flavonoid supplementation may benefit the critical period of executive function development in children between the ages seven to ten. This may lead to greater maturation of executive function skills at this age with a secondary benefit to school performance, such as for reading behaviour, as well as a potential reduction in the prevalence of learning and behavioural disorders.

1.1.1.4.4.1 Episodic memory

An initial cross-over study by Whyte and Williams (2015) first demonstrated that an acute 100 ml flavonoid-rich BB treatment (143 mg anthocyanins) significantly improved delayed word recall on the Rey's Auditory Verbal Learning Task (AVLT) in eight to ten-year olds compared to baseline. The same improvements were not observed for the placebo.

Although Whyte and Williams (2015) failed to observe effects on executive function, a finding consistently observed in the adult literature, intake of the acute BB treatment was shown to benefit delayed word recall in this child population. In general, declarative memory tasks are sensitive to the effects of WBB supplementation as demonstrated by research in adults. However, it is important to note that no further significant effects were seen for any other AVLT outcome measures, or for the tasks assessing working memory or executive function which were shown to improve following acute supplementation in adults. One explanation is that the changes to the other cognitive domains of interest are too subtle to be detected acutely in such a small sample size (*N* = 14), therefore cognitive improvements on the other domains assessed here cannot be ruled out entirely.

Following on from their original study, Whyte et al. (2016) aimed to replicate their initial findings and to investigate the relationship between dose, post prandial test point, and cognitive performance in seven to ten-year olds (*N* = 21). A randomised, double-blind, placebo controlled, acute cross-over design was employed with children consuming a low flavonoid 15 g WBB treatment (125 mg anthocyanins), a high flavonoid 30 g freeze-dried WBB powder treatment (253 mg anthocyanins), or a sugar-matched placebo; each treatment was administered on individual test days separated by a one-week wash out period. On the individual test days, cognitive performance was assessed by a test battery: word recall and recognition (AVLT), executive function (Modified Flanker Task; MFT), response inhibition (Go-NoGo), and levels of processing and response interference (Picture Matching Task; PMT). Parallel versions of the cognitive tasks were completed at baseline, 75 minutes, three hours, and six hours post-treatment on all four test days. Consistent with Whyte and Williams (2015), significant effects of acute WBB consumption were observed for measures of word recall and recognition, with an increase in final acquisition performance at 75 minutes post-consumption following intake of the 30 g WBB treatment. Although performance on the word recognition component of this task declined from baseline following all treatments, this decline was significantly greater for those consuming the placebo at six hours, compared to consumption of the 30 g WBB treatment. This suggests that acute intake of the high-flavonoid WBB treatment attenuated the natural decline in word recognition performance observed at six hours following the placebo. This indicates a maintenance of performance effect following WBB supplementation over this period of time.

1.1.1.4.4.2 Executive function

Initial research by Whyte and Williams (2015) failed to observe acute BB effects on executive function. It was thought to be a result of the small sample size obtained and the palatability of the treatment. Here, participants may have struggled to consume the whole treatment, as well as negative responses to the taste of the BB treatment consequently having an adverse effect on cognitive performance. However, the follow-up study by Whyte et al. (2016) which addressed these issues observed significant improvements to executive function accuracy for trials of both low and high cognitive demand three hours after consumption of the 30 g WBB treatment compared to the placebo. This finding suggested that, in addition to general improvements to accuracy performance on the lower cognitive demand trials, the 30 g WBB treatment may have benefitted children in times of high cognitive demand. In particular, the high-flavonoid WBB treatment may have aided performance when greater demand was placed on cognitive resources, helping children to overcome response interference effects and to complete the task. This was consistent with performance on the PMT which assessed levels of processing and response interference. Here, performance on the more cognitively demanding name-match trials was greater at all time points following intake of the 30 g WBB treatment compared to trials requiring less cognitive demand. In line with the previous study, it is important to note that only a minority of cognitive domains were susceptible to the effects of acute WBB supplementation and not all outcome measures of the cognitive tasks were shown to benefit from the WBB treatments. These were the first two studies to assess the effects of a WBB treatment on cognition in children.

With initial research (Whyte et al., 2016) demonstrating an acute effect of WBB supplementation on executive function, in particular on the more cognitively demanding trials, Whyte et al. (2017) set out to test differing levels of cognitive demand on executive function performance in seven to ten years olds after acute WBB supplementation. In line with the previous studies, children consumed both a 30 g freeze-dried WBB powder treatment (253 mg anthocyanins) and sugar-matched placebo, and were tested on the Modified Attention Task (MANT) three hours after treatment. This task allows the manipulation of cognitive demand by changing the degree of difficulty of the task parameters (stimuli congruency, stimuli load, stimuli target time). Therefore, certain trials become more cognitive demanding than others as they require greater cognitive resources

in order to complete them correctly. In this instance, executive function reaction times were significantly faster for the more cognitively demanding trials three hours after WBB supplementation compared to placebo; intake of the WBB treatment maintained and directed attention to the more cognitively demanding incongruent, high load trials. These trials required greater attention in order to overcome the distracting stimuli to produce a correct response. This follow-up indicated enhanced executive function reaction times for trials of high cognitive demand following an acute WBB treatment. Further, the findings implied that sensitivity and demand of the task also played a vital role in the level of performance achieved and can also be influenced positively by acute WBB supplementation.

Although limited, the research in children so far positively demonstrates the effects of acute WBB interventions on the cognition of seven to ten-year olds, in particular for the domains of word recall and recognition, and executive function. These are the first studies to highlight the benefits of berry-flavonoids on cognition in children. It is important to replicate this research to gain a comprehensive understanding of the cognitive domains acute WBB consumption can significantly improve. Future replication is also important to determine whether these positive benefits can extend to other cognitive domains not yet tested, or to other related behaviours such as mood and reading behaviour. Mood is thought to moderate cognition (Section 1.1.1.5) whilst reading behaviour is thought to rely upon the cognitive domains (Section 1.2) already known to be susceptible to WBB supplementation in adults and more recently in children.

In summary, there is a range of epidemiological and RCT research examining the acute and chronic effects of berry-flavonoids on cognition across the human life span. Initial preclinical trials demonstrate robust significant improvements to spatial working memory in both young and older rodents with and without neuropathology (for review, see Rendeiro et al., 2012). Stemming from these preclinical findings, significant berry-flavonoid effects have been observed consistently for episodic memory, working memory, and executive functions in young and older adults with and without neuropathology (for review, see Lamport et al., 2012; Bell et al., 2015; Hein et al., 2019). These significant effects are observed following both acute and chronic treatments. Most recently, research has started to examine the effects of acute berry-flavonoid consumption on cognition in children, with positive effects observed for word recall and recognition, and executive functions (Whyte and Williams, 2015; Whyte et al., 2016; 2017).

Table 1.1.1.4.4: Summary of studies examining the relationship between anthocyanin consumption and cognitive performance in children.

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Whyte & Williams (2015)	Four female, ten male mean age 9.17 years	200 g fresh 'Star' variety blueberries, 100 ml semi-skimmed milk, 8 g sucrose (143 mg anthocyanins). Placebo was matched for sugars and vitamin-C added to 100 ml semi-skimmed milk	Acute, randomised, double-blind, placebo-controlled, cross-over with a seven day wash out	Tested at baseline and two hours. Go-NoGo, AVLT, Stroop test, n-back task, Object Location Task	Blueberry had significantly greater long delayed recall 25 minutes after presentation compared to placebo. Blueberry had significantly less proactive interference compared to placebo
Whyte et al. (2016)	Twelve female, nine male mean age 8.7 years	15 g freeze-dried whole fruit wild blueberry powder (127 mg anthocyanins, 30 g freeze-dried whole fruit wild blueberry powder (253 mg anthocyanins) both added to 170 ml water and 30 ml low energy fruit squash (Rocks brand). Placebo and 15 g treatment matched to the 30 g treatment for sugars and vitamin-C	Acute, randomised, double-blind, placebo-controlled, cross-over with a seven day wash out	Tested at baseline, one hour fifteen mins, three hours and six hours. AVLT, ANT, Go-NoGo, PMT	30 g WBB had significantly greater final acquisition word recall at one hour 15 mins compared to placebo. 15 g WBB attenuated the decline in long delayed word recall at all time points compared to placebo and 30 g WBB. Both treatments attenuated the decline in word recognition at six hours. 30 g WBB had significantly greater ANT accuracy for the more cognitively demanding incongruent trials three hours. Similarly, 30 g WBB had significantly faster PMT responses for the more cognitively demanding named-match trials.

Table 1.1.1.4.4: *continued…*

Author	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Whyte et al. (2017)	Ten female, eleven male mean age 8.11 years	30 g freeze-dried whole fruit WBB powder (253 mg anthocyanins) added to 170 ml water and 30 ml low flavonoid orange cordial (Rocks Orange Squash). Placebo matched for sugars vitamin-C	Acute, randomised, double-blind, placebo-controlled, cross-over with a seven day wash out	Tested at baseline and three hours. MANT	30 g WBB had significantly faster MANT responses to 500 ms slow trials compared to placebo and 120 ms fast trials. £0 g WBB had significantly faster MANT responses to the more cognitively demanding high load trials. 30 g WBB had significantly faster MANT responses for cue presentation in general.

1.1.1.5 Berry-flavonoids and improvements to mood

Most recently, research has started to examine the effects of berry-flavonoids on mental health and wellbeing as the need for alternative interventions is on the rise compared to the limited availability of psychotherapeutic and pharmacological interventions. Berry-flavonoids are hypothesised to be one possible cost-effective and naturally occurring intervention to reduce symptoms associated with mood disorders (Khalid et al., 2016). Research has demonstrated a link between the pathways involved in cognition and mood within the frontal cortex. Indeed, a commonly reported symptom of depression is impaired cognitive functioning, specifically executive function; deficits to executive functions are attributed to certain depressive symptoms such as low mood and negative thoughts (Marazziti et al., 2010). To date, not one underlying mechanism of action of berry-flavonoids has been highlighted to singularly contribute to the cognitive and mood changes associated with mental health. Rather, a number of mechanisms have been put forward to potentially explain what is happening within the brain to elicit such changes following berry-flavonoid intake. Berry-flavonoids have been shown to increase CBF, a finding associated with improvements to cognitive performance (Dodd et al., 2019; Bowtell et al., 2017) and the amelioration of low mood or depressive states (Schore, 2016). Berry-flavonoids have also been shown to moderate monoanime oxidase (MAO) inhibition (Dreiseitel et al., 2009), a process vital for efficient cognitive functioning and regulation of the mood hormones serotonin, dopamine and noradrenaline. Last, berry-flavonoids act by modulating Gamma Amino Butyric Acid (GABA), an inhibitory neurotransmitter involved in the regulation of cognition, with low levels thought to be associated with mood disorders (Hanrahan et al., 2011).

As previously noted, berry-flavonoids have been shown to benefit cognition across the human lifespan, and similar benefits to mood are starting to become apparent. Evidence from epidemiological studies demonstrates that a greater lifetime consumption of fruits and vegetables, and hence a higher intake of flavonoids in general, predicted a lower incidence of depression in later life (Mihrshahi et al., 2015; Chang et al., 2016). In addition, a systematic review by Khalid et al. (2016) which set out to examine the relationship between children/young people (18 years and under) with internalising disorders, such as low mood and anxiety, and nutrition, demonstrated a positive association between consumption of a diet rich in flavonoids and lower levels of depression. To date, there is limited research

examining the effect of berry-flavonoids on mood, with a variety of inconsistencies amongst those that have been conducted: source of flavonoids, duration of intervention, sample recruited, mood state measured (fatigue, alertness, positive and negative affect) as well as the results obtained.

Acute intervention of a blackcurrant extract and blackcurrant juice (matched polyphenol concentrations) on the cognition and mood of healthy young adults (Watson et al., 2015; Section 1.1.1.4.4.3) observed significant cognitive improvements post-consumption of both treatments, independent of mood effects. Here, no effects of treatment consumption were apparent for mood when assessed via a Bond-Lader visual analogue scales (VAS; Bond & Lader, 1974). This null effect is supported by Whyte et al. (2019; Section 1.1.1.4.4.3) whereby supplementation of the acute 400 ml mixed berry treatment (254.65 mg anthocyanins) did not affect either positive affect or negative affect, as assessed by the Positive Affect and Negative Affect Scale-NOW (PANAS-NOW; Watson et al., 1988) in healthy young adults. In addition, a further lack of mood effects following chronic 24 g/d BB (19.2 mg/g anthocyanins) was observed in healthy older adults (Miller et al., 2019; Section 1.1.1.4.4.3). No changes in mood or depressive state, as measured by the Profile of Mood States (POMS; McNair et al., 1981) and Geriatric Depression Scale (GDS; Yesavage, 1988), were observed over a 90-day period.

Thus far the findings detailed present a non-existent effect of berry-flavonoids on mood in healthy adults. However, these studies were powered in order to observe cognitive effects primarily, not mood. Therefore, with appropriately powered studies, mood effects may potentially be observed following berry-flavonoid interventions. Nevertheless, research is starting to emerge which does observe significant improvements to mood following berry-flavonoid supplementation. Khalid et al. (2017) observed a significant increase in self-reported positive affect, as measured by the PANAS-NOW, two hours after an acute 30 g freeze-dried WBB treatment (253 mg anthocyanins) in young adults (aged 18-21 years), compared to placebo. Most recently, a pilot study by Watson et al. (2019) examined the effect of an acute blackcurrant juice (500 mg total polyphenols) on mood and attention determined by the use of electroencephalography (EEG) to assess changes to prefrontal cortex neuronal activity. The study highlighted an anxiolytic effect following intake of the blackcurrant juice, compared to placebo. This was in line with a reduction in α spectral power and an increase in slow wave δ and θ spectral powers. Further EEG increases in β

power and reductions in α spectral power indicated greater alertness and lower fatigue. These changes to mood were accompanied by a significant increase in digit span short-term memory.

The studies presented here report the use of several different mood measures, each measuring a different aspect of mood and thus failing to provide a standardised and generalisable measure of mood. The VAS, POMS and PANAS-NOW provide a measure of self-reported emotional or mental state in that precise moment of testing. However, the mood states of interest can be different for each study. For example, Watson et al. (2015) employed 16 VAS scales to assess participants' mood on a number of state outcomes (drowsy, excited, etc…). On this occasion, the scores from each scale were then combined to form three mood factors to be analysed: alert, calm, content. This was in line with Bond and Lader (1974) where factor analysis conducted on the original 16 scales identified three mood factors which shared 61.1% of the overall variance: alertness, calm, contentedness. Therefore, the 16 scales were allocated to one of these three identified mood factors depending on which they had the highest loading. However, by grouping the VAS scores into three mood factors for analysis, this has the potential to mask effects seen for individual items. Indeed, a similar approach was used by Miller et al. (2019) whereby 50 individual affect items were grouped into six mood factors of interest. Before combining the scales into mood factors, it would have been prudent for Watson et al. (2015) and Miller et al. (2019) to have conducted their own factor analysis to examine mood factor unity between the scales used. This would have indicated whether it was appropriate to analyse the data as combined mood factors. Indeed, it may be possible that the 16 scales loaded onto different mood factors or onto none at all.

In addition, both Watson et al. (2015) and Miller et al. (2019) seemed to measure arousal state rather than mood *per se*, such as positive affect or negative affect as measured by the PANAS-NOW, thus still not providing a consistent result on whether flavonoid-rich treatments can benefit mood. The limited literature investigating the effects of berry-flavonoids on mood thus far remains inconclusive and further investigation is needed utilising a single, standardised measure of mood. The literature demonstrating the shared neural mechanisms between cognition and mood highlights a possible link between cognition, mood, and flavonoid consumption.

In summary, there is limited research examining the effect of berry-flavonoids on mood in humans, with research conducted thus far providing inconsistent findings. Whilst the majority of research fails to show a change to mood following berry-flavonoid treatments, although significant improvements to executive function are apparent, research is starting to emerge which demonstrates positive benefits to mood. Further research is needed to examine the effects of berry-flavonoid interventions on mood. As stated above, research demonstrates shared activation of the prefrontal cortex for both executive function and mood, highlighting a potential association between mood effects and executive function effects.

1.2 Reading

In order for research to examine the effects of berry-flavonoids on reading behaviour in children, it is first important to understand what reading is, how this process develops in children, and the cognitive domains involved in reading. The sections below aim to answer these questions and to set up a background framework on the potential of berry-flavonoids to improve reading behaviour in children.

1.2.1 What is reading? - The Simple View of Reading

Reading is the multifaceted process by which readers can construct meaning from printed text. Successful reading requires the identification of words from text (decoding) in parallel with the ability to construct an understanding of the words read (language comprehension). The involvement of decoding and language comprehension makes up the basis of The Simple View of Reading (Gough & Tunmer, 1986), where reading equals the product of decoding and comprehension. Although decoding and language comprehension both contribute to reading individually, neither is sufficient on its own. For example, an individual who cannot decode a word will not be able to read. However, the single act of reading a word does not guarantee successful reading until the individual can construct meaning from the words being read.

As stated by The Simple View of Reading (Gough & Tunmer, 1986), the foundation of reading is built upon the abilities of decoding and language comprehension. The coordination of both decoding and language comprehension allows for a more automatic and accurate approach to reading, vital for fluent reading. These processes pave the way for more complex reading skills such as reading comprehension; this is commonly demonstrated

by the high correlation between word reading and reading comprehension whilst children are in the initial phases of reading development (Cain, Oakhill & Bryant, 2004). Therefore, limited word reading ability will impact upon reading as a whole (Torgesen, Rashotte & Alexander, 2001).

The Simple View of Reading provides a useful framework for thinking about reading. However, it is not without limitations. For example, it does not acknowledge a multitude of other factors that contribute to reading, for instance motivation, background knowledge or individual differences in vocabulary knowledge (Perfetti & Stafura, 2014). In addition, the Simple View of Reading does not discuss, in depth, the learning of individual words and the processes by which this can be achieved, or how reading development commences. The research within this thesis focuses specifically on word reading and associated reading behaviours, therefore the remainder of this literature review will focus on word reading.

1.2.2 Theories of skilled word reading

The literature proposes a number of theoretical frameworks for how word reading can be achieved. The Dual Route Model of reading aloud (Coltheart et al., 1993; Figure 1.2.3A) proposes two routes for the reading of words: the lexical route for fast visual recognition of familiar words and the non-lexical route for deciphering novel words. The lexical route enables the identification of a previously learnt word, with key information relating to that word such as orthography (visual form), phonology (spoken form), and semantics (meaning) extracted from the internal lexicon. When an unfamiliar word is encountered, the lexical route cannot be used to identify the word. As this word is new to the reader, the reader's internal lexicon holds no information specific to that word to help with its identification; consequently, the non-lexical route is activated. Here, rules relating to English letter-sound correspondences allow the reader to break down or 'decode' the unfamiliar word into its individual graphemes (letters and letter combinations) before converting them to phonemes based on grapheme-phoneme conversion rules. The novel word is then reassembled to provide the reader with a correct pronunciation which is then stored for future retrieval.

In sum, novel words can only be deciphered via the non-lexical route whilst known words can be read by either the lexical or non-lexical route. However, there are some deviations. Exception words such as *yacht* or *colonel* fail to follow typical letter-sound correspondences. Here, use of the non-lexical route for exception words such as *yacht* or *colonel* would yield an incorrect pronunciation (e.g., yacht to rhyme with matched). In these instances, word reading is only possible via the lexical route whereby pre-existing word representations from the internal lexicon are used to aid exception word reading. In order to become a skilled reader, the reader must develop an adaptable word reading system to accommodate words with varying regularity in letter-sound correspondences.

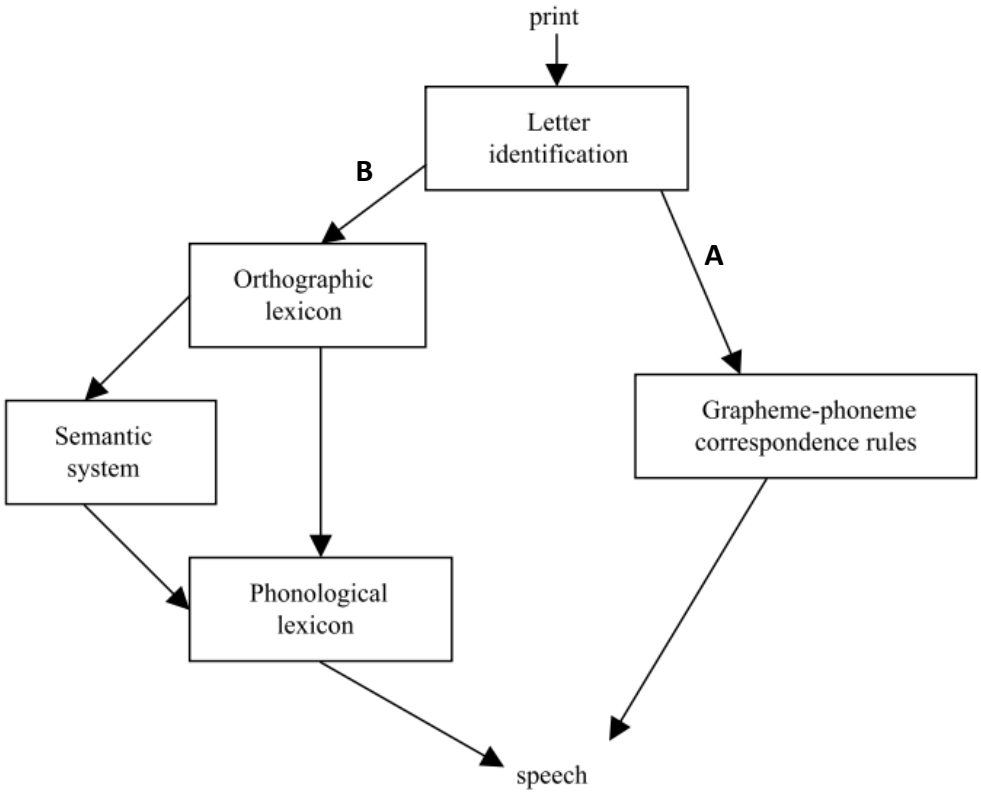


Figure 1.2.2A: The Dual Route model of reading aloud depicting **A)** the lexical route and **B)** the non-lexical route. Schematic from Coltheart, 2006.

Whilst the Dual Route Model of reading aloud encompasses the use of two routes for word reading, triangle models suggest that dual processes are not necessary to read both novel and known words (Seidenberg, 2005). Triangle models (Harm & Seidenberg, 2004; Plaut et al., 1996; Seidenberg & McClelland, 1989; Figure 1.2.3B) denote that all words, whether regular or irregular, are processed via a single mechanism comprising three

components: phonology, orthography, and semantics. A series of feedforward and feedback associations between these components are learnt over the course of reading development. These associations are weighted and encompass the statistical properties of all words the system has encountered; typically words encountered most frequently have greater weighted associations between these components.

Triangle models generally display two pathways for reading a word: the phonological pathway mapping associations between phonological and orthographic information, and the semantic pathway mapping associations between semantic, phonological, and orthographic information. The phonological pathway produces a word's pronunciation through decoding attempts, whilst the semantic pathway produces a pronunciation through the activation of the word's meaning. Essentially, all three components work together with the sum total of all knowledge used to produce the pronunciation of an encountered word. Overall, the connections emphasised by triangle models become strengthened through repeated exposure to words, improving the rapidity and accuracy of word reading.

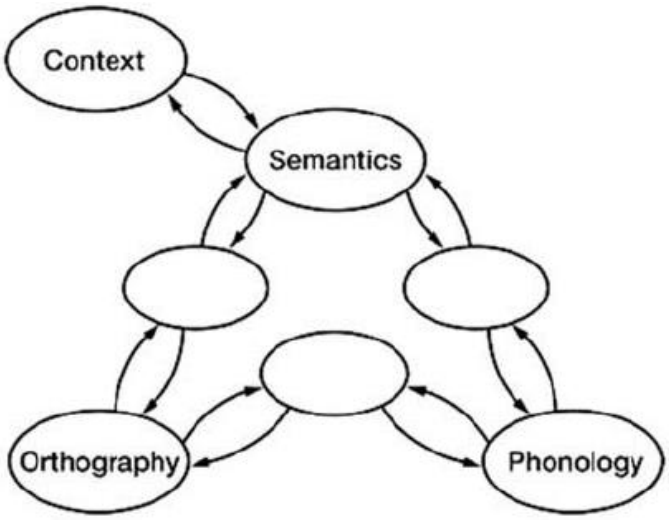


Figure 1.2.2B: Schematic adapted from Seidenberg and McClelland's (1989) general framework for word processing. Each oval represents a group of units and each arrow represents a group of connections. 'Context' here refers to contextual factors derived from syntactic, semantic, and pragmatic constraints which are thought to influence the construction of the sematic level which therefore indirectly influences construction of representations at the other levels.

Both the Dual Route Model and triangle models posit the involvement of phonological decoding and whole word recognition in successful reading. Whole word recognition is only available to individuals who have learnt to read some words, and phonological decoding only to individuals who have learnt the relationships between letters and sounds. The next section will consider how children learn letters and sounds and to read words.

1.2.3 Learning to read words

Before children become adept sight word readers, Ehri (1995) states all children start off in the pre-alphabetic phase with no concept of how to read or knowledge of the alphabet. Here, pre-readers start to recognise words from the surrounding environment such as from signs and symbols through repeated exposure. Letter-sound correspondences are not considered during this phase, instead all connections between environmental print and words are formed through contextual cues and visual features (Ehri & Snowling, 2004). It is not the words themselves that are recognised, but rather the context in which they are found (Masonheimer et al., 1984). Through these experiences, beginner readers start to learn the sounds associated with letters within words.

When learning to read words, all readers should first understand the alphabetic principle which teaches that letters of the alphabet and the phonemes connected to them are used to read words (typically taught through phonics instruction). Once these connections between graphemes and phonemes become established, the reader is able to slowly decode words using a letter-by-letter approach. Indeed, without this knowledge and the subsequent ability to decode, children are unlikely to make sense of printed text. There are exceptions where reading known words can be achieved without decoding, such as through visual memory, although given the number of words that share common letters and letter strings, high cognitive demand would be placed on visual memory systems to achieve this. Although the reading of known words without decoding is possible by making use of visual memory systems, this does not extend to non-words. Therefore, this suggests that the reading of known words without decoding relies on learnt associations between printed and spoken words (Burne, 2014) such as those encountered in Ehri's (1995) pre-alphabetic phase.

Once children begin to learn the link between phonemes and graphemes, they transition to the partial alphabetic phase. Here, the first and last letters of a word become crucial. As only parts of a word can currently be recognised, readers struggle to fully decode unfamiliar

words although their limited letter-sound correspondences do enable memories for those successfully decoded. During this phase, reading by analogy can aid with novel word reading. As proposed by Ehri (1995), reading by analogy uses information provided by a similarly spelt word previously encountered and stored in the internal lexicon to understand the unfamiliar word's letter-sound correspondences and to produce the correct pronunciation. Goswami (1986) suggests that reading by analogy is an emerging strategy in the early phases of reading development as children are able to read words correctly if they share similar beginning and ending letters. Although, some decoding skills are still needed to detect these similarities suggesting analogy might not be for complete beginners (Ehri & Robbins, 1992); this process is not as strenuous as whole word decoding. Once readers gain a full knowledge of the alphabet and are able to form complex connections between phonemes and graphemes, they move into the full alphabetic phase (Ehri, 1995).

As these connections between phonemes and graphemes develop in the full alphabetic phase, spellings and pronunciations become fully bonded in memory and can be automatically retrieved, signalling the development of fast and efficient sight word reading. Indeed, Ehri and Wilce (1987) found that children who had received full alphabetic training were able to process all grapheme-phoneme connections, aiding their sight reading for a group of similarly spelled words. Those who did not receive training, and hence were still classed as partial alphabetic readers, showed difficulty in recognising the individual words due to their lack of fully formed grapheme-phoneme connections.

As reading development progresses, Ehri (1995) suggests that children adopt more and more sophisticated strategies of learning to read words dependent on their experience with print and reading in general. Contextual support can be used by skilled readers when a novel word is encountered. Here, prediction from context allows the reader to predict what the unfamiliar word may be, leading to more efficient and fluent reading of the remaining passage and minimising the disruption to reading comprehension. However, it is argued that reading through prediction of context is not supportive of establishing an enhanced lexicon or word recognition skills (Jenkins, Matlock & Slocum, 1989), and that the success of this method relies upon the reader's initial vocabulary suggesting this strategy is used by more skilled readers with an established vocabulary. As a reader's skill set develops further, readers advance into the last phase of reading development; consolidated alphabetic phase.

In the consolidated alphabetic phase (Ehri, 1995), all the previous skills developed throughout the framework now become refined to maximise an individual's reading ability and minimise disruption. Beyond the previous processes of word learning mentioned (phonological recoding, reading by analogy, prediction from context), readers are able to draw upon more advanced strategies such as using rimes and affixes to aid in the reading of simple and complex words, such as multisyllabic words. Sight word reading is crucial in this phase as reading development turns to focus on reading comprehension.

Another influential theory of learning to read is the self-teaching hypothesis (Share, 1995) which suggests that effortful decoding supports orthographic learning. As decoding commences, readers create orthographic representations of newly encountered words and commit them to memory. This helps grow the visual lexicon, the store of sight vocabulary that is essential for achieving text-reading and comprehension. When encountered again in written text, the orthographic representation of a word will automatically activate memory of that word from the internal lexicon, bringing to mind its pronunciation and other useful related information. This demonstrates two pathways for reading; one for reading familiar words and one for decoding novel, unfamiliar words.

Both Ehri's (1995) phase development and Share's (1995) self-teaching hypothesis fit succinctly with the word reading strategies outlined by the Dual Route Model of reading aloud (Colheart et al., 1993) and triangle models (Harm & Seidenberg, 2004; Plaut et al., 1996; Seidenberg & McClelland, 1989). The ability to decode a novel word to produce its correct pronunciation maps directly onto the strategies employed by the non-lexical route of the Dual Route Model and that of the orthography to phonology pathway of the triangle models. The correct pronunciation of the novel word is then committed to the internal lexicon for future retrieval upon recognition of the word when next read. Here, the self-teaching hypothesis describes how the sublexical route of the Dual Route Model of reading aloud aids the development of the internal lexicon in order to make the lexical route more effective. The retrieval of all key information related to a known word upon recognition maps onto the lexical route of the Dual Route Model, via the sublexical route, and the orthography to semantic pathway of the triangle models. Further, the nature of the word to be read determines which strategies are used. Novel words and exception words are read through the non-lexical route or through the employment of all three components in the triangle models, whilst know words are read through the lexical route and orthography to

semantics pathway. The more advanced route/pathway used to read known words also allows for the use of reading strategies such as reading by analogy and reading through contextual support where a wider vocabulary is necessary.

1.2.4 Strategies for improving word reading

Literacy skills are important for educational progression in childhood and become the foundation for adult educational and occupational success. It is therefore surprising that approximately 17% of UK adults have low literacy proficiency, a finding mirrored in other predominantly English-speaking countries: Australia 17%, Canada 16%, and Northern Ireland 17% (Department of Business Innovation and Skills, 2013). This highlights the importance of interventions during childhood to prevent poor literacy skills in adulthood, a concept deemed more cost-effective than later interventions (Heckman & Masterov, 2007). To this extent, children are taught reading and literacy using phonics methods through the national curriculum. The current strategies for improving reading in children focus on phonics instruction and phonological awareness training (Duff & Clarke, 2011). Both strategies underpin the development of reading by teaching children the basic foundational skills necessary; perceiving and manipulating the phonology of words.

Deficits in phonological decoding can severely impact reading development in children, therefore strategies and teaching techniques are needed to aid word learning in order for progression to take place. Research demonstrates that children who receive phonological awareness training, such as learning to categorise words based on their pronunciation, show superior phonological skills on a range of measures involved in the awareness of syllables, rimes and phonemes (Bradley & Bryant, 1985; Lundberg et al., 1988; Duff & Clarke, 2011); an effect reported to last for several years (Byrne & Fielding-Barnsley, 1995). Phonological awareness training not only elicits positive effects through oral training, but also when combined with letter training, providing a connection between the phonology and orthography of written language. This method benefits typically developing children (Bus & van Ijzendoorn, 1999), as well as those at risk of reading difficulties (Ehri et al., 2001b). However, a report by the National Reading Panel (2000) showed that only 6.5% of the variance in children's reading ability could be attributed to phonological awareness training, increasing to 10% when letter knowledge was included. Although benefits are exhibited, this strategy alone may not be sufficient enough to aid reading in children.

Unanimously, the most beneficial approach for teaching word reading is through systematic phonics instruction (Castles et al., 2018). This approach teaches the relationships between graphemes and phonemes within an alphabetic writing system, such as English, providing readers with the ability to decode and access the meanings of words. Synthetic phonics programs teach grapheme-phoneme correspondences (GPC) individually and in a specified sequence before teaching children to blend individual phonemes together to make a word. Analytic phonics programs start with whole words. Here, GPC are taught by segmenting a word into its individual parts. Both approaches are akin to early theories of reading development such as the self-teaching hypothesis (Share, 1995) where the experience of encountering a novel word and being able to decode it allows the storage of that word in the internal lexicon for later retrieval. In addition, they further map onto the Dual Route Model and triangle models of word reading where the reading of known words and novel words activate the appropriate routes or pathways for efficient decoding and subsequently word reading. Although it would seem that phonics and phonological awareness have advantages over each other; for example, as implemented by synthetic phonics programs, introducing individual GPC in the beginning allows for more controlled GPC learning optimising correct sequence learning, both approaches are equally weighted in their effectiveness of teaching word reading (Torgerson et al., 2006; Ehri et al., 2001). All that is required is that the program is systematic, teaching GPC in an ordered manner. Indeed, the effectiveness of this approach to teaching word reading was adopted by the English school curriculum after the Rose (2006) review, and performance from phonics screening checks highlights year-over-year improvements since 2012 in seven to eleven-year olds (U.K. Department for Education, 2016).

However, phonics is only effective if children have an initial understanding of phonological awareness. In this capacity, Torgesen (2005) suggested that in order for reading interventions to be effective, they should consider both phonological awareness and phonics. Shapiro and Solity (2016) examined the effect of phonological awareness on the effectiveness of synthetic phonics programs for teaching word reading. Overall, phonological awareness was significantly related to all reading measures. However, a greater positive relationship was observed between phonological awareness and exception word reading when utilising a *Letters and Sounds* synthetic phonics program. This program teaches children multiple GPC, compared to the *Early Reading Research* synthetic phonics program

which teaches only consistent GPC mappings as well as frequent words by sight. Therefore, the direct learning of multiple GPC was better able to improve exception word reading with children who had a firm understanding of phonological awareness. Further, analysis of interventions including both phonological awareness training and phonics showed that children progressed faster with their word reading and reading comprehension. Nevertheless, some children still fail and struggle with these methods (Torgesen, 2000). Further investigation is needed to understand why these children do not respond to such strategies, and how these strategies can be adapted to suit their needs (Al Otaiba & Fuchs, 2006; Duff et al., 2008).

1.2.5 Nutrition and academic performance

One potential strategy for improving reading in children is the use of dietary interventions. As detailed above (Section 1.1.1.4), acute WBB treatments have been shown to significantly improve aspects of cognition such as episodic memory, working memory, and executive functions, all known to underlie reading processes. As noted by Whyte and Williams (2015), these positive findings highlight the potential for WBB treatments to benefit performance in a real-world educational scenario, such as reading. The section below (Section 1.2.6) provides further support for the use of dietary interventions on improving reading.

Initial research started to examine the effects of dietary interventions on academic performance as a means of expanding findings from cognition-based test batteries to real-world educational scenarios. To date, a variety of dietary interventions have been employed with a wide range showing improvements to academic performance post-treatment. Multivitamin and mineral (MVM) interventions have, in general, been shown to significantly increase reading speed, learning capacity, arithmetic and non-verbal intelligence in healthy children (Benton et al., 2001; Schoenthaler et al., 2000; Wang et al., 2003). One such study by Wang et al. (2003) who supplemented eight to twelve-year olds with a MVM supplement for six months found significantly greater performance in reading speed, learning capacity, and arithmetic examinations compared to a placebo. These behavioural changes were parallel with higher urine excretion of vitamins B2 and C, higher serum concentrations of 25-OH-D3 (vitamin-D) and greater bone mineral content. Additionally, habitual breakfast consumption in children is positively associated with improved academic performance with

greatest effects observed for mathematics and arithmetics particularly in undernourished individuals (for a review, see Adolphus et al., 2013). Similarly, habitual breakfast consumption in adolescents was more likely to result in higher mathematics GCSE grades compared to those who rarely consumed breakfast (Adolphus et al., 2019). However, results from such studies are often inconsistent as each study uses academic measures specifically standardised and established in their country of origin so cannot easily be generalised to children in other countries. Additionally, a variety of academic measures are used between the studies (reading, arithmetic), making it hard to identify and distinguish the consistent effects of nutrition on academic performance.

Research demonstrates a number of links between better executive function and academic performance in general. For example, greater working memory and inhibition are found to be predictive of greater maths and reading ability in nine to twelve-year-old school children (Gerst et al., 2017; St. Clair-Thompson & Gathercole, 2006). Further, a positive association was observed between greater processing speed and greater performance for maths, reading ability and language skills in six to nineteen-year olds (Geary, 2011). These links between executive functions and academic performance, reading in particular, further highlight the potential for berry-flavonoid supplementation to improve reading behaviour in children. Alongside the findings that acute WBB intake positively benefit aspects of cognition that are involved in both academic performance and reading behaviour, these preliminary acute findings highlight the potential for chronic WBB supplementation to improve reading behaviour and academic performance over a longer period of time. This proposition is in line with the chronic dietary intervention studies on academic performance reported above (Section 1.2.5).

1.2.6 Nutrition and reading

Recent evidence suggests that dietary interventions may be an effective strategy to benefit reading. Research examining the effect of diet on reading ability has primarily focused on the impact of long-chain omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The results from such studies have reported significant improvements to reading and cognition in children with specific developmental conditions: attention-deficit/hyperactivity disorder and developmental coordination disorder (Milte et al., 2011; Richardson, 2006; Richardson & Montgomery,

2005). Here, Milte et al. (2011) reported a significantly positive correlation between EPA/DHA levels and word reading ability in children with ADHD; higher levels of EPA and DHA were associated with greater word reading performance on the word reading sub-test of the Wechsler Individual Achievement Test 3rd Edition (WIAT-III) in comparison to controls of the same age. The task assessed children's word reading ability, reading comprehension, and pseudo-word decoding, thus ultimately assessing the final product of learning to read such as when children reach the full alphabetic stage of reading where sight word reading becomes established. Similarly, Richardson and Montgomery (2005) observed that children with developmental coordination disorder supplemented for three months on a fish oil capsule containing omega-3 and omega-6 fatty acids increased their reading age by nine months when assessed by the Wechsler Objective Reading Dimensions task (a predecessor of the WIAT-III). This was in comparison to those receiving the placebo where reading age only increased by three months. This increase was still apparent at a six-month follow-up whereby those receiving the treatment showed an improved reading age of thirteen months compared to those receiving the placebo who maintained the three-month improvement in age. These findings suggest that PUFA supplementation in those with learning difficulties and developmental conditions can help performance improve following chronic supplementation. One explanation is that children with learning difficulties and developmental conditions have a habitual diet low in PUFA levels and as a result are more susceptible to PUFA supplementation. This could explain the prevalence of positive findings in this cohort of children who have a dietary deficit, not in typical children who may already be consuming a diet sufficient in PUFAs. However, such findings from clinical populations cannot be generalised to the performance of healthy mainstream school children.

Research examining the impact of PUFAs in typically developing children has started to emerge. An epidemiological study by Zhang et al. (2005) examined the relationship between six to sixteen-year olds' dietary intake of PUFAs and their performance on a reading task (Wide Range Achievement Test); letter recognition, word recognition, and sentence comprehension were assessed and scores combined to give an overall reading composite score. It was found that those with a higher intake of PUFAs had a significantly lower probability of poor reading performance compared to those with a low intake. Similarly, low omega-3 levels were associated with lower than average reading performance as assessed by the Word Reading Achievement sub-test of the British Ability Scale (BAS; Montgomery et

al, 2013). In support of the epidemiological evidence, an RCT by Richardson et al. (2012) examined the effect of DHA supplementation (600 mg per day) over sixteen weeks on reading, working memory and behaviour in healthy, but underperforming children. Reading was assessed via the Word Reading Achievement sub-test of the BAS. Here, children were presented with a list of words from simple monosyllabic orthography to harder multisyllabic orthography assessing their previous knowledge of letter-sound correspondences and potential sight word reading of already known words. The study found that those who performed 10-20% below that of age-matched peers at baseline showed a greater improvement in their reading after receiving the DHA treatment. Similarly, Dalton et al. (2009) found that spelling and reading performance were maintained six months post-consumption of a fish-flour spread (containing DHA and arachidonic acid; AA) compared to a decrease seen for the placebo condition as supported by a trend. Here, reading was assessed by the Hopkins Verbal Learning Task assessing word recognition. However, a recent parallel-groups, placebo-controlled study which aimed to replicate Richardson et al. (2012)'s study failed to observe significant improvements to reading, working memory or behaviour in underperforming seven to nine-year olds following sixteen-week DHA supplementation (Montgomery et al., 2018). This finding is disappointing when considered in line with the positive benefits of PUFA supplementation on reading behaviour in typical children (Richardson et al., 2012; Dalton et al., 2009). Therefore, this small collection of studies highlights the need for future replication to gain a consistent understanding of the effects of PUFA supplementation on reading behaviour in typical children.

To date, the role of berry-flavonoids on reading behaviour has not yet been examined. However, as reported above, research does demonstrate improvements to word list recall and recognition, and executive functions, following both acute and chronic berry-flavonoid treatments. These findings highlight the potential for berry-flavonoids to benefit children's reading behaviour, specifically the visual retrieval of words stored in their internal lexicon, and to executive functions thought to play a vital role in word learning and reading comprehension (Sesma et al., 2009; Christopher et al., 2012). One key difference to note between the reading literature and the cognition/flavonoid literature is terminology. The flavonoid/cognition literature denotes word learning as the recognition of known list of words, whilst the reading literature portrays word learning in its true form: the learning of novel words. This is an important distinction to note when moving forward with the research

in this thesis. So far, the acute and chronic WBB effects have been observed for word list reading where improvements in the numbers of words recalled or recognised have been considered. In order for research to examine the effects of WBB treatments on word learning, novel words would need to be used. This would allow the direct assessment of the effects of WBB supplementation on the decoding of the novel words, the process by which words are learnt. Going forward, the research within this thesis will examine word reading of known words using measures of word list recall and recognition, as well as word learning through the inclusion of novel words. Indeed, non-words will be used for measures of word learning as they allow for the assessment of pure spelling-sound knowledge without interference from known words.

1.3 The present research

From the literature review above, it is apparent that berry-flavonoids have the potential to elicit cognitive benefits following both acute and chronic supplementation across the human lifespan, however findings relating to children are limited and require further investigation. Recent work from Whyte and Williams (2015) and Whyte et al. (2016; 2017) provides a positive framework detailing the acute benefits of WBB supplementation on word list recall and recognition, and executive function up to six hours post-consumption. Nevertheless, use of subtly different methodologies between the three studies highlights the need for replication in order for consistency amongst results.

In Chapter 2, Study 1 aimed to replicate Whyte et al.'s (2016) research by examining whether an acute WBB treatment could positively benefit the cognition of seven to ten-year olds. In addition to cognition, the study examined whether the cognitive benefits seen previously by Whyte et al. (2016) could extend to reading behaviour; known to rely upon aspects of cognition such as working memory and executive function, that have previously been shown to benefit from an acute WBB treatment. For consistency and interpretability this study employed the same measures of executive function, word recall and recognition as Whyte et al. (2016), with the addition of a word reading task to assess whether acute WBB consumption could elicit further improvements to reading behaviour. The acute effect of WBB supplementation on mood were further assessed. All testing took place in the afternoon to fit in around the normal school day (see Section 2.2.5).

In Chapter 3, Study 2 aimed to examine whether time of day impacted the effect of acute WBB on cognition, reading behaviour, and mood. The rationale for examining time of day is fully described in Chapter 3, however, to summarise, it was necessary to investigate inconsistencies in the findings between Study 1 and the wider literature (Whyte et al., 2016). The same study procedure, cognitive test battery, and treatments were used, but with children tested in the morning. The data sets from Study 1 and Study 2 were then combined to examine the effect of time of day on performance.

In Chapter 4, Study 3 aimed to examine the chronic effects of daily WBB treatment over four weeks on cognition, reading behaviour, and mood in seven to ten-year olds. To date the chronic effects of berry-flavonoid supplementation on children's cognition has not been examined. Taking into account the positive benefits to children's cognition seen acutely, it was possible that chronic WBB supplementation would either maintain these positive benefits or elicit greater benefits accruing over time due to the cumulative effect of WBB following continued supplementation. In addition, benefits to word reading were most likely to be observed following chronic WBB supplementation. Given the complexities of word reading, it is possible that an acute dose may not be sufficient to produce measurable changes. Therefore, the study employed an acute-on-chronic design, with both acute and chronic test sessions, to examine differences between acute (baseline, two hours post-treatment test sessions) and chronic test sessions (two weeks, four weeks test sessions).

Finally, Study 4 detailed in Chapter 5 aimed to examine how children learn to read words, and whether this ability was impacted by an acute WBB treatment. More specifically, the study focused on eight to nine-year olds' (Section 5.2.1) abilities to learn to novel words, and subsequently learning of the important mappings between a word's written form (orthography) and pronunciation (phonology), as well as the acute effects of WBB intake on the retention of newly learnt words over a 24-hour period.

1.3.1 Summary of thesis aims and hypotheses

The overall aims of the present research detailed in this thesis are:

- To examine the acute effects of WBB supplementation on the episodic memory and executive functions, reading behaviour, and mood of seven to ten-year olds.

- To examine whether there is an interaction between time of day (morning vs afternoon) on the acute effects of WBB supplementation on the episodic memory and executive functions, reading behaviour, and mood of seven to ten-year olds.

- To examine the chronic effects of daily WBB supplementation over four weeks on the episodic memory and executive functions, reading ability and mood of seven to ten-year olds.

- To examine the acute effects of WBB supplementation on the ability to learn novel words in eight to nine-year olds.

The overall hypotheses predicted by the present research detailed in this thesis are:

- That acute WBB treatment will significantly improve episodic memory, executive functions, and mood two hours after treatment, with greater improvements seen for more cognitively demanding executive function trials (Studies 1 & 2).

- That acute WBB treatment will have an impact on reading behaviour two hours after treatment (Studies 1 & 2).

- That the effects of acute WBB treatment on episodic memory and executive functions, reading behaviour, and mood, will differ dependent on time of day (Study 2).

- That chronic WBB treatment will significantly improve episodic memory, executive functions, and mood over a four-week period, with greater improvements seen for more cognitively demanding trials (Study 3).

- That chronic WBB treatment will have an impact on reading behaviour over a four-week period (Study 3).

- That acute WBB treatment will have an impact on word learning when assessed at two hours and 24 hours after treatment (Study 4).

Chapter 2

Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds.

Mood data from this Chapter has been published as: Khalid, S., Barfoot, K. L., May, G., Lamport, D. J., Reynolds, S. A., & Williams, C. M. (2017). Effects of acute blueberry flavonoids on mood in children and young adults. *Nutrients, 9*(2), 158.

Cognitive data from this Chapter has been published as: Barfoot, K. L.*, May, G.*, Lamport, D. J., Ricketts, J., Riddell, P. M., & Williams, C. M. (2018). The effects of acute wild blueberry supplementation on the cognition of 7-10 year-old schoolchildren. *European Journal of Nutrition,* 1-10.

* joint first authors

The role of other researchers in this chapter: K L Barfoot undertook 50% of data collection.

2.1 Introduction

As summarised in the previous chapter, the cognitive benefits from acute and chronic berry-flavonoid interventions are seen consistently amongst adults (for a review see Bell et al., 2015; Lamport et al., 2012). Most recently, research has started to consider whether such benefits can extend to child populations; a novel target population.

Pioneering the research with children, Whyte and Williams (2015) and Whyte et al. (2016; 2017) initially showed that acute wild blueberry (WBB) supplementation could benefit the cognition of seven to ten-year olds. Consistent with the positive benefits observed in healthy adults (Bell et al., 2018; Dodd et al., 2019), significant improvements to children's episodic memory were observed. Initial research demonstrated a significant improvement to delayed word recall two hours after intake of an acute freeze-dried blueberry (BB) powder treatment (143 mg anthocyanins; Whyte & Williams, 2015). This finding was supported by subsequent research demonstrating significantly improved immediate word list recall performance at 75 minutes after intake of an acute 30 g freeze-dried WBB powder treatment (253 mg anthocyanins; Whyte et al., 2016). Further, a maintenance in word recognition performance was seen at multiple time points up to six hours after intake of this WBB treatment, suggesting a maintenance of word recognition performance over the six-hour period. Together, these findings highlight that acute WBB interventions can positively benefit word

list recall and recognition up to six hours after treatment consumption. However, both these studies employ interventions with differing concentrations of anthocyanins and demonstrate a range of findings on different aspects of word list recall and recognition. Although both studies observe findings for the same cognitive outcomes at different time points after administration, this is not unexpected. It is important to note that the effects of acute WBB supplementation on cognition may not necessarily occur at the same time after treatment due to the biphasic response of BB flavonoids on flow-mediated dilation. Rodriguez-Mateos et al. (2013) observed peaks in BB flavonoid metabolites within the bloodstream at one to two hours, and six hours after consumption of a BB treatment (766 mg total BB polyphenols). However, despite this biphasic response of BB flavonoids, the other differences between the two studies noted above highlight the importance of replicating the above word list recall and recognition findings to establish which areas of episodic memory are reliably and consistently benefitting from acute WBB interventions.

Similar improvements following acute WBB supplementation have been observed for executive function performance. Whyte et al. (2016) were the first to observe significantly greater accuracy on more cognitively demanding Modified Flanker Task (MFT) trials at three hours after intake of the 30 g WBB treatment. This finding was in line with a significant improvement on a Picture Matching Task (PMT), also assessing executive function, with better performance observed again for the more cognitively demanding trials. Most recently, Whyte et al. (2017) showed a significant improvement to executive function reaction time following supplementation of an acute 30 g freeze-dried WBB powder treatment. Specifically, acute WBB intake exhibited faster reaction times on the more cognitively demanding Attention Network Task (ANT) trials three hours after consumption, suggesting greater sustained attention was directed to the harder trials resulting in greater inhibition of the distracting stimuli.

These findings relating to executive function suggest that acute WBB supplementation may benefit children during times of high cognitive demand. Despite such benefits, there remains a discrepancy between the two studies. The former research by Whyte et al. (2016) exhibited an improvement to executive function accuracy, whilst the later exhibited an improvement to executive function reaction time (Whyte et al., 2017) on slightly different tasks of executive function. Although conceptually related, these tasks differ in their level of cognitive demand. The MFT requires participants to respond to a central arrow presented

for 120 ms. This arrow is surrounded by flanker arrows that are either congruent (i.e. <<<<< or >>>>>) or incongruent (i.e. <<><< or >><>>) in direction. Only the duration between arrow presentation was altered (1000 ms, 1300 ms, 1500 ms). In contrast, the ANT manipulated the cognitive demand of the task between trials, specifically altering the speed of arrow presentation (120 ms or 500 ms), the number of arrows presented (1, 5, 10), as well as congruency (Section 2.2.4.2). Therefore, this is one explanation for the discrepancy noted. This discrepancy is not novel, as findings from the adult literature highlight an inconsistent effect of berry-flavonoids on the executive function outcome measures of accuracy and reaction time, often affecting one outcome measure but not the other on a range of executive function tasks. For example, Watson et al. (2015) observed the slowing of executive function reaction times for those receiving a blackcurrant extract on a digit vigilance task, following continuous repeated cognitive testing. However, this slowing in reaction times was attenuated for those receiving blackcurrant juice which maintained reaction times over the repeated digit vigilance task. In contrast, accuracy performance on the rapid visual information processing task decreased over repetitions after consumption of the blackcurrant juice, whilst intake of the blackcurrant extract attenuated this decline. Although compositional differences in the juice versus the extract may be responsible for the differences in executive function performance (Watson et al., 2015), this does not explain why such differences were apparent for Whyte et al.'s (2016; 2017) research; the same intervention was accountable for both accuracy and reaction time improvements on the conceptually similar executive function tasks. However, the variety of executive function measures used may be responsible for the differing results. As Whyte et al.'s two studies alone make up the berry-flavonoid research in children thus far, it is important to conduct future research to fully understand which aspects of executive function are consistently benefitting from acute WBB interventions in this target population.

2.1.1 WBB and reading behaviour

The positive benefits of acute WBB supplementation on children's cognition may have the potential to extend to a real-world educational scenario, such as reading behaviour. Reading involves the complex mental coordination of many elements: phonology, orthography, semantics, strategies for reading comprehension (Pressley, 2006), all of which must work simultaneously to achieve fluent reading and reading comprehension (Cartwright, 2009; Section 1.2). These map onto aspects of executive function which develop in parallel with

reading throughout early childhood and into adolescence. Here, prereading skills are related to inhibition (Blair & Razza, 2007) and cognitive flexibility (Farrar & Ashwell, 2008). For example, Blair and Razza (2007) found that the inhibitory control of three to five-year olds, when assessed by a peg-tapping task, was significantly related to their development of important prereading skills such as phonemic awareness and letter knowledge. Word-reading proficiency is related to working memory (Welsh et al., 2010), shifting, and updating (van der Sluis et al., 2007). Indeed, van der Sluis et al. (2007) found that composite scores of shifting and updating were significantly related to word reading efficiency in nine to twelve-year olds. Last, reading comprehension is associated with planning, working memory (Sesma et al., 2009; Locascio et al., 2010), and inhibition (De Beni & Palladino, 2000), to name a few. Sesma et al. (2009) observed that nine to fifteen-year olds classed as poor comprehenders had significant deficits to planning and working memory, whilst De Beni and Palladino (2000) observed significant deficits in eight-year old poor comprehenders' abilities to inhibit irrelevant information, even with adequate word reading ability. These associations suggest that executive function influences successful reading and thus highlights that berry-related executive function improvements in children may have a secondary impact on their reading behaviour.

Indeed, research demonstrates associations between executive function skills and important reading skills throughout childhood. Altemeier et al. (2008) found that inhibitory control in nine to ten-year olds significantly predicted their word reading development, suggesting that word reading relies upon inhibition to omit distracting information and to allow for the successful decoding of a target word. Working memory is associated with phonological awareness and was shown by Welsh et al. (2010) to predict later reading skills such as word and non-word reading, as well as memory for content. Further, processing speed as assessed by the Colorado perceptual speed (CPS) test 1-2 and the ETS identical pictures task, was shown to be a significant, independent predictor of word reading ability, specifically fluency (Christopher et al., 2012).

Considering the above literature, the present study investigated the effect of an acute WBB treatment on the speed and accuracy of word reading. The Test of Word Reading Efficiency (TOWRE-2; Section 2.4.2.4) was employed to assess children's efficiency of reading known words (sight word efficiency), and non-words to specifically assess decoding ability for novel orthography (phonemic decoding efficiency). Both sight word reading of known

words and reading of non-words are reliant upon executive functions (Wagner et al., 1997; Deater-Deckard et al., 2009). Research has demonstrated positive associations between executive functions, such as effortful control, and word and non-word reading; these associations were particularly strong in elementary school aged children (eleven to sixteen years). For example, Christopher et al. (2012) demonstrated positive correlations between both working memory and processing speed for word reading. This suggests that sight word reading uses both working memory and processing speed for the efficient retrieval of information about a previously encountered word. Phonemic decoding may rely upon working memory to hold a non-word's orthography in mind whilst drawing upon a range of decoding skills to produce a correct pronunciation, before committing the newly learnt word to memory.

2.1.2 The present study

A between groups, randomised, single-blind, placebo-controlled study design was implemented to extend findings from Whyte and Williams (2015) and Whyte et al.'s (2016; 2017). Participants were randomised to receive either an acute 30 g freeze-dried WBB powder treatment or a placebo. Measures of word list recall and recognition, executive function, reading behaviour, and mood, were completed at baseline and two hours after treatment. To align this study with the previous research (Whyte et al., 2016), a target population of seven to ten-year olds were recruited. At this age, children experience a critical period of development where aspects of cognition, such as executive functions (cognitive flexibility, working memory, inhibition) and episodic memory, improve markedly (Cartwright, 2009; Davidson et al., 2006). In addition, it is between the ages of six to ten years that the teaching of reading moves from the use of phonics and reading simple words and sentences, to accurate and fluent reading ability, vital for reading comprehension.

Taking the previous research into account (Whyte & Williams, 2015; Whyte et al., 2016; 2017), the present study hypothesised that an acute 30 g freeze-dried high-flavonoid WBB treatment would improve word list recall and recognition, and executive function two hours after treatment, compared to a placebo. Secondly, it was hypothesised that acute WBB supplementation would aid performance on more cognitively demanding executive function trials, in comparison to less cognitively demanding trials and compared to a placebo. This is consistent with Whyte et al.'s (2016;2017) findings (Section 2.1). Last, the study

hypothesised that intake of the acute WBB treatment would positively affect both reading behaviour and mood two hours after treatment, compared to a placebo.

2.2 Methods

2.2.1 Participants

For the present study (for ethical approval see Appendix A.1), an *a priori* power analysis (using G Power 3.1) based on the significant AVLT total word recall finding from Whyte et al. (2016) was used to calculate the required sample size. A two-tailed test with a partial alpha of p = .05 revealed a total of 50 children would be required to achieve sufficient power of 0.8 (n = 25 per treatment group) when employing a between-subjects design.

A total of 54 healthy children (25 male: 29 female) aged seven to ten years (M = 8.24, SD = .97) were recruited from two primary schools local to the University of Reading. Written consent (see Appendices B.1 & C.1) was obtained from parents/legal guardians prior to participation, as well as participant's assent (see Appendices D.1 & E). Parents/legal guardians confirmed their child spoke English as a first language, had no known learning or behavioural difficulties (e.g. ADHD, dyslexia or reading impairments), and had no fruit or fruit juice intolerance. All 54 participants completed the study in full.

2.2.2 Background measures

Participants completed a series of background measures during an initial practise session: the children's version of the Raven's Coloured Progressive Matrices (RCPM; Raven, 1965) as a measure of non-verbal intelligence, and the Continuous Performance Task (CPT; Conners, 1994) as a measure of sustained attention. The York Assessment of Reading for Comprehension (YARC; Snowling et al., 2009; see Appendix G) measured reading accuracy, reading rate and reading comprehension, and the Single Word Reading Task (SWRT) sub-test of the YARC measured word reading accuracy (see Appendix H). Raw scores and standard scores for these tasks are shown in Table 2.4.1.

2.2.2.1 Raven's Coloured Progressive Matrices (RCPM; Raven, 1965)

An easy to administer and an easy to interpret test of non-verbal intelligence widely used in both practical and research settings. Designed for use in children aged five to eleven years, the RCPM incorporates a series of 36 printed diagrams divided into three sets (twelve per set) of increasing difficulty which all have a part missing. Participants are required to

complete the design by selecting the correct missing piece from six printed options. Every correct response resulted in a score of 1 point, with a maximum score per set being twelve points or 36 points in total across all three sets. The diagrams and items used in the RCPM are appropriate to measure performance in children able to sufficiently reason by analogy and who have adopted this strategy as a consistent method of inference.

The RCPM is known to be sensitive to fluctuations in intellectual function, demonstrating good test-retest reliability (r = .80; Cotton et al. 2005). The RCPM has been shown to correlate well with other measures of non-verbal intelligence for use in children such as the Wechsler Intelligence Scale for Children (WISC). Evans (1980) noted that seven to eight-year olds showed a positive correlation of r = .56 between performance of the two test (significant at the $p < .01$ level), whilst nine to ten-year olds showed a positive correlation of r = .65 (significant at the $p < .01$ level). This suggests the RCPM has a good level of validity for accurately measuring non-verbal intelligence in children, as well as having a high level of ecological validity (Raven, 2000).

2.2.2.2. Continuous Performance Task (CPT; Conners, 1994)

The CPT is a long-established test used for measuring vigilance, response inhibition, and signal detection in a vast range of individuals of all ages. In general, participants are required to press a key in response to a specific target, usually the letter X. For this collection of studies, an adapted version of the CPT was created for easier use in children. Participants were presented with coloured circles and were asked to respond all coloured circle, but not when presented with an orange circle. The CPT produces a standard set of outcome measures such as percentage commission errors (failure to make a response to a circle other than orange) and percentage omission errors (failure to respond to an orange circle), of which both were used to measure general sustained attention throughout this research.

Split half reliability for all CPT outcome measures ranged between r = .73 and r = .96 and test-retest reliabilities for these outcome measures over a three-month period range between r = .55 and r = .84 (Conners, 2000), suggesting adequate reliability. The validity of the CPT at present is controversial. To date, research has evaluated the validity of the CPT by comparing performance on the measure between typical children and those with inattentive and hyperactive-impulsive behavioural disorders. Edwards et al. (2007) failed to observe correlations between CPT parameters and behavioural ratings in six to twelve-year olds who

were identified as having attention deficits. However, Berger et al. (2017) observed that seven to twelve-year olds with ADHD had significantly worse performance on four CPT parameters: attention, timing, hyperactivity, and impulsivity, compared to a control group without ADHD. Receiver operating characteristic analysis revealed fair to excellent diagnostic ability of all CPT parameters, highlighting appropriate validity of the measure and suggesting that the CPT is sufficient for attentional disorder diagnosis. Although these findings demonstrate inconsistencies regarding the CPT's validity, the CPT is not affected by ethnicity or gender, rendering it a useful tool to assess sustained attention in schools.

2.2.2.3 York Assessment of Reading for Comprehension (YARC; Snowling et al., 2009)

The YARC is a standardised assessment of reading comprehension used for both educational and research purposes. The Passage Reading version of the YARC was designed for use within primary school children aged five to eleven years to assess the reading fluency, reading accuracy, and reading comprehension of oral reading skills. Participants were required to read aloud a passage matched to their grade level which was determined by the Single Word Reading Sub-test; a simple test of general vocabulary knowledge. Afterwards, participants were asked eight open-ended comprehension questions. If five or more of these questions were answered correctly, the participant was then asked to read a passage one level higher than the starting passage, followed by the comprehension questions for this passage. However, if participants answered four or less of the comprehension questions correctly from the initial passage, they were asked to read a passage one level lower than the starting passage. Participants were required to read aloud two passages in total. Passages were a mixture of fiction and non-fiction, and participants read one of each type. In addition to obtaining reading comprehension scores for each passage reading accuracy errors and reading rate were also recorded.

YARC reading accuracy and reading rate were determined as having high reliability of estimates due to relative ease at which these constructs can be measured (reading accuracy: r = .75+, reading rate: r = .91+). In contrast, YARC reading comprehension was determined to have a lower reliability of estimates (ranging from r = .48 to r = .77). This is to be somewhat expected as reading comprehension is a multi-faceted construct and comprehension scores were obtained from a small number of questions. However, by participants completing two passages, this increased the reliability of estimates range for the reading comprehension

construct to *r* = .71 to *r* = .84. All three YARC constructs were thought to have high validity due to their exact testing of decoding ability (reading accuracy), fluency (reading rate) and literal and inferential comprehension.

The YARC produces initial raw scores for each participant, such as the number of correctly answered comprehension questions or the number of reading accuracy errors made. Each participant achieved two sets of raw scores for each of the YARC constructs measured, one from each of the two passages read. The raw scores cannot be compared directly between participants because children of different ages and abilities read different passages. Therefore, raw scores were converted to standard scores to allow comparison across the sample. In order to obtain standard scores for each participant, the two sets of raw scores for each construct were combined and converted into an average ability score. This is an estimate of each participant's reading skill and reflects both the raw scores and difficulty of the passage. The average ability scores were obtained using the Rasch model, an arbitrary numbering system used to express performance in a metric fashion. The ability scores were then converted to standard scores using the tables provided in the YARC manual (Snowling et al., 2009) to show each participant's standing in relation to a sample of participants of the same age. The YARC gives standard scores in the range of 70 – 130, with an average standard score being 100 to follow a normal distribution. Scores outside of this range are classed as extreme outliers.

2.2.3 Treatments

An acute single-blind, randomised, between-subjects design was applied with participants randomly allocated to receive either a 30 g freeze-dried WBB powder treatment or a sugar-matched placebo (*n* = 29 WBB, *n* = 25 placebo). The 30 g WBB treatment was equivalent to 240 g or one and a half cups of fresh WBB, containing 253 mg anthocyanins and 766 mg total polyphenols (108 kcal). The placebo contained fructose (8.9 g), glucose (7.99 g) and vitamin-C (4 ml) to match the concentrations found in the 30 g WBB treatment. To aid consumption and palatability, 170 ml of cold tap water and 30 ml of a low-flavonoid orange squash (Rocks brand, UK) were added to both treatments, giving a total of 200 ml in liquid to consume. Treatments were prepared by the researchers immediately prior to consumption, and participants consumed the treatment in an opaque drinking flask using an opaque straw. Participants were given a maximum of five minutes to consume the treatment.

2.2.4 Cognitive measures

2.2.4.1 Rey's Auditory Verbal Learning Task (AVLT; Lezak, 1983; see Appendix I)

This task measured word learning through word list recall, memory and recognition. Participants heard an auditory recording of 15 nouns (list A), read at one per second, for a total of five presentations per test session. Each presentation was followed by a free recall of this list (recalls A1-A5). A new list of 15 nouns (list B) was read aloud as an interference list on trial six and was recalled once only (recall B). Participants then recalled list A after a short delay (two minutes; recall A6) and a long delay (25 minutes; recall A7). After recall A7, participants were visually presented with 50 nouns containing: words from lists A and B, and 20 additional nouns. They were asked to circle words from list A only to assess word recognition. Parallel versions of the original word list (Appendix I) were used to avoid practise effects (Crawford et al., 1989) and were counterbalanced across test sessions. The parallel versions were of equivalent difficulty, matched for: word length, the number of syllables, frequency.

For each test session, a series of outcome measures were calculated according to Lezak et al. (2004) and previous research (Whyte & Williams, 2015): immediate word span (recall A1); words learnt (recall A5 – recall A1); final acquisition (recall A5); proactive interference (recall A1 - recall B; the effect of prior learning of List A on the recall of List B); retroactive interference (recall A5 – recall A6; the effect of learning List B on the prior recall of List A); total recall (recall A1 to recall A5 summed); short delayed recall (A6); long delayed recall (A7); total delayed recall (recall A6 + recall A7), and word recognition (the number of words correctly circled).

2.2.4.2 Modified Attention Network Task (MANT; Fan et al., 2002; Whyte et al., 2017)

This task measured executive function, specifically response interference and response inhibition. This task combined a cue-target and flanker task to measure vigilance, selective attention and response interference under conditions of differing cognitive demand. Here, stimuli load, target presentation time, orientation and cueing were manipulated to change the cognitive demand of the task. In accordance with Whyte et al. (2016), a practise block of 35 trials was initially completed, where target duration decreased from 1000 ms to 120 ms over 16 trials to allow familiarisation with the speed of response required. Subsequently, participants completed four target blocks of the MANT each consisting of 80 trials.

For the main target blocks, a fixation slide was first shown with a cross displayed centrally. This was followed by either a cue or no cue for 120 ms with cue location (above or below the central fixation cross) randomised between trials. The target stimulus, a single arrow pointing “<” or “>”, was then displayed either above or below the fixation cross for a time of either 120 ms (two target blocks) or 500 ms (two target blocks); stimulus location was not always consistent with prior cue location. The target stimulus appeared individually or surrounded by pairs of flanker arrows, and could be congruent (i.e. <<<<< or >>>>>) or incongruent (i.e. <<><< or >><>>) with the surrounding arrows; these were always orientated in the same direction. Individual arrows exhibited low load whilst congruent or incongruent trials either exhibited medium load (one row of five arrows) or high load (two rows of five arrows of the same congruency). Target time was either 120 ms (fast trials) or 500 ms (slow trials). The stimulus position, congruence and load were randomised between trials, displayed in equal probability with each 80-trial block consisting of: 32 congruent trials, 32 incongruent trials, 32 high load trials, and 32 medium load trials. The remaining trials in each block consisted of low load single arrows (Figure 2.2.4.2). Participants were instructed to press the left or right arrow key on the keyboard according to the direction of the target stimulus arrow for each trial.

The influence of MANT parameters congruency, load, and target time on the outcome measures of response accuracy (number of correct hits) and response reaction time for correct targets only (speed of response to target) were investigated. Reaction times <100 ms were removed to control for responses made before the stimuli could be properly processed by the participants (Whyte et al., 2017).

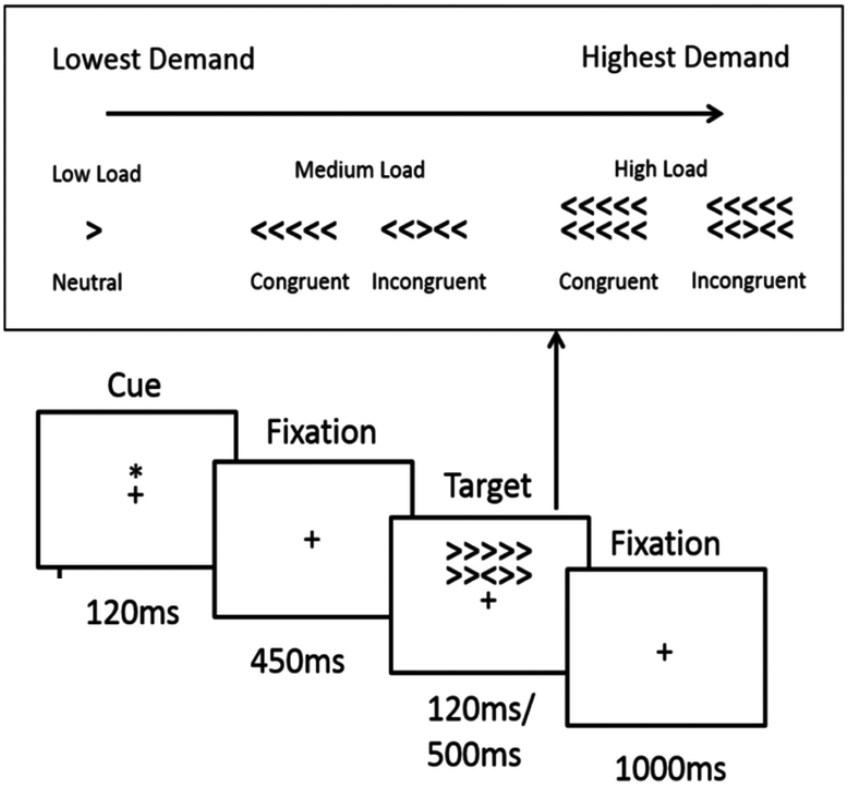


Figure 2.2.4.2: Schematic of the Modified Attention Network Task (MANT; Whyte et al., 2017) detailing the cognitive demand of the MANT parameters: congruency (congruent, incongruent), load (medium, high) and target time (120 ms, 500 ms), as well as the presentation of stimuli to participants.

2.2.4.3 Positive and Negative Affect Scale for Children (PANAS-C; Laurent et al., 1999; see Appendix K)

This was used to measure current mood and is a validated child friendly version of the PANAS-NOW and PANAS-X questionnaires (Watson et al., 1988; Crawford & Henry, 2004) normed in nine to twelve-year olds. The measure has good convergent and divergent validity (Chorpita & Daleiden, 2002); for example, the negative affect scale demonstrated greater associations with criteria measuring anxiety and depression than the Affect and Arousal Scales. Further, the PANAS-C met Nunnally and Berstein's (1994) cut-off for good internal consistency for both positive affect ($\alpha = .89$) and negative affect ($\alpha = .92$) as determined by Cronbach's alpha of > .80. The PANAS-C consisted of 15 positive affect (PA) and negative affect (NA) items or associated emotions/feelings. For example, negative affect items within the questionnaire consisted of 'sad,' 'disgusted' and 'anxious' whilst positive affect items consisted of 'happy,' 'cheerful' and 'lively'. Participants were asked to rate how they felt at

that moment for each item on a Likert scale ranging from one to five, with one being “Not at all” to five being “Extremely.”

The outcome measures for the PANAS-C were PA and NA, calculated by summing positive items and negative items as per standardised instructions (Laurent et al., 1999).

2.2.4.4 Test of Word Reading Efficiency (TOWRE-2; Torgesen et al., 2012; see Appendix J)

The TOWRE-2 is a measure of reading fluency and accuracy of known and unknown words, normed in six to 24-year olds. The TOWRE-2 showed a high degree of reliability at *r* = .99 for all four forms examining its sensitivity to: scorer differences, delayed administration, test-retest reliability. Further, the TOWRE-2 is considered to have a high degree of construct validity as has been shown to correlate highly with a number of other measures of reading fluency and accuracy. For example, Kuhn (2005) observed a correlation of *r* = .84 for sight word reading efficiency in typical readers and those identified as poor readers (aged eleven to sixteen years) when comparing the TOWRE-2 and Oral Reading Fluency. Mathes et al. (2005) observed a high correlation of *r* = .83 for phonemic decoding efficiency in typical readers and those identified as poor readers (aged eleven to sixteen years) when comparing the TOWRE-2 and Woodcock-Johnson III.

The test consisted of two subtests: sight word efficiency (SWE) and phonemic decoding efficiency (PDE). The SWE subtest measured word reading efficiency, whilst the PDE subtest measured non-word reading efficiency. For both, participants were visually presented with words (108) or non-words (63), increasing in difficulty from single-syllabic to multi-syllabic, and had to read as many items as possible in 45 seconds. Each subtest had four parallel forms which were randomised between participants and sessions.

The outcome measures for the TOWRE-2 were number of words (SWE) and non-words (PDE) pronounced correctly in 45 seconds.

2.2.5 Procedure

2.2.5.1 Practise session:

Participants took part in an initial practise session one to two days prior to the start of testing. Here, participants completed a practise of the AVLT and MANT to ensure complete understanding of the task to eliminate practise effects. Participants further completed the

background measures mentioned previously: non-verbal IQ, sustained attention, general reading ability, and single word reading accuracy. To avoid the items used in the YARC and SWRT interfering with the practise of the AVLT task, these background measures were completed last. After completion of the practise session, participants were asked to follow a 24-hour low-flavonoid diet (see Appendix F) prior to testing. An information sheet detailing high-flavonoid foods to avoid (berry-fruits, general fruit and vegetables, chocolate, etc) and some low-flavonoid alternatives was given to parents/legal guardians to ensure compliance. Parents of participants were called 24 hours prior to the test day to remind them of compliance with the low flavonoid diet.

2.2.5.2 Test day:

Testing took place during the afternoon after lunch with participants tested individually in a quiet space at their schools. Participants consumed either a low-flavonoid packed lunch provided by parents or a low-flavonoid cooked lunch provided by the school canteen on the day of testing. Further, participants were asked whether they had conformed to the 24-hour low-flavonoid diet prior to testing. The specific contents of the lunch were not standardised or recorded. Lunch was consumed in the 75 minutes period prior to baseline testing. All participants completed an initial baseline session at either 1200 hours, 1245 hours or 1330 hours immediately before consuming the WBB treatment or placebo. After consumption, all children returned to class for a two-hour period where they were only allowed to consume water and were asked to abstain from exercise. Cognitive performance was assessed at two hours after treatment consumption for consistency with Whyte et al. (2016) and to coincide with the time at which berry-flavonoid metabolites are known to peak within the bloodstream: biphasically at one to two hours and six hours post-consumption (Rodriguez-Mateos et al., 2013). The post-treatment test sessions were, therefore, at either 1440 hours, 1525 hours or 1610 hours (dependent on time of consumption). Here, participants completed matched versions of the same test battery to assess any changes in performance. Test versions of equivalent difficulty were counterbalanced across visits and treatment groups. During each test session, participants completed the tasks in the following order: PANAS-C, AVLT recalls A1-A6, MANT, AVLT recall A7, word recognition, and TOWRE-2 (Figure 2.2.5.2). Each session lasted approximately 40 minutes.

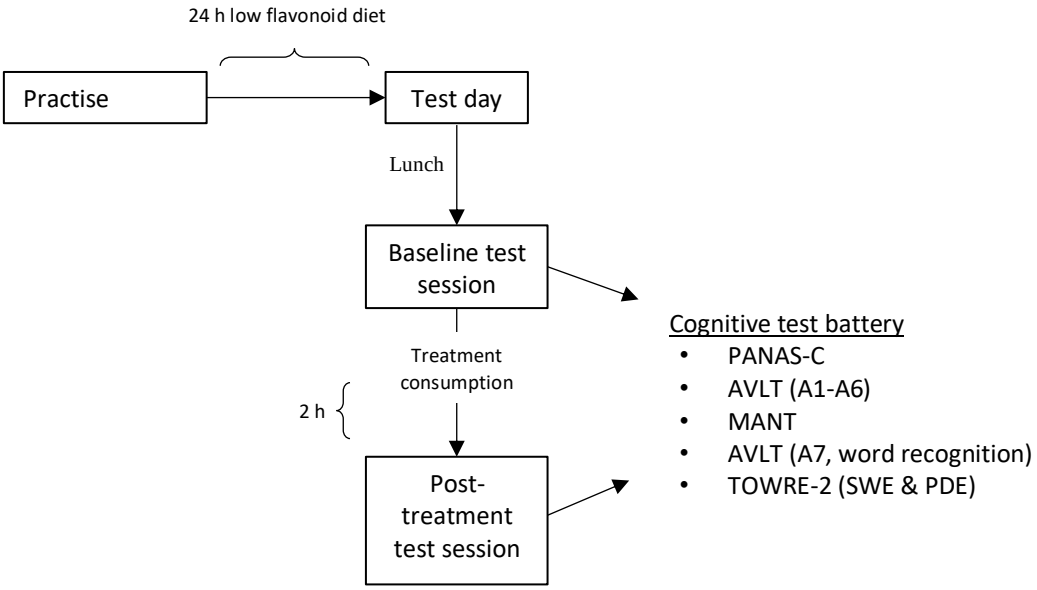


Figure 2.2.5.2: Schedule of test sessions, intervention, and task order.

2.3 Statistical analysis

Data were analysed using SPSS (Version 21.0). Differences between treatments (WBB, placebo) for baseline variables: non-verbal IQ, CPT omissions and commissions, general reading ability, and SWRT, were examined using independent-samples t-tests. Two chi-square tests were performed to assess sex differences across the treatment groups. Means, standard deviations and group comparisons are presented in Table 2.4.1.

Linear Mixed-effects Models (LMM) using unstructured covariance matrices were used to analyse data, with a separate model for each post-treatment dependent variable: all AVLT outcome measures (word span, words learnt, final acquisition, proactive interference, retroactive interference, total recall, short delayed recall, long delayed recall, total delayed recall, word recognition), MANT accuracy and reaction time (ms; correct responses only), both TOWRE-2 SWE and TOWRE PDE, and both PANAS-C PA and PANAS-C NA.

LMM were employed for the present study as are advantageous over the use of other statistical approaches such as ANOVA. First, unlike for ANOVAs, where all effects and interactions are included in order to run the analysis, regardless of whether they are hypothesised or not, LMM allow for a simpler, but more flexible format where only effects and interactions of interest are included in the model. This approach is known as confirmatory analysis and increases the power of the analysis in comparison to other

statistical approaches, such as ANOVA, by not wasting degrees of freedom on unwanted variables and comparisons. By using confirmatory analysis, the researcher can clearly set out to examine the exact variables of interest.

Second, although one of the standardised assumptions of such statistical analyses is the independence of data points, which is not always appropriate for repeated measures data sets, LMM can overcome the issue of independence by the inclusion of random effects. Random effects, such as participants and items (for example, the different MANT trials would each count as an individual item, due to having their own variance around the by-item mean) give structure to the error term. These help to explain what else is contributing to the variance of the model, aside from the fixed effects already included. For example, including participants as a random effect, allows the researcher to control for individual differences amongst participants throughout the data set. LMM can account for both random effects of participant and item during the same analysis, however with other statistical programmes such as ANOVA, only one random effect can be accounted for at any time whilst the other is disregarded.

Last, LMM are useful when data points are missing. Although this is mostly directed at repeated measures designs where there are multiple data points per participant, LMM allow the analysis to continue by only excluding the one data point that is missing rather than deleting all the participant's data, as would be done when using ANOVA (listwise deletion). This can further cause a reduction in statistical power and creates a bias in the sample. As LMM do not require balanced data, the power of the analysis increases when participants with missing data points need not be excluded completely.

In general, LMM adopt the same assumptions as other statistical approaches such as ANOVA: linearity, absence of collinearity, homoskedascity, normality of residuals, and independence of data points. Therefore, the current models run in the present study broadly assume that the outcomes of all dependent measures are the product of a linear combination of independent variables, meaning that no two independent variables should correlate which can lead to problems with cause and effect, amongst others. The data points should show no extreme or disadvantageous skewing and preferably sitting within one standard deviation of the mean; the same principles must also apply to residuals whereby all are expected to be evenly distributed. Last, LMM assume independence of data points,

however as stated above, this assumption is not always appropriate for the type of analysis being conducted and can be overcome when needed, through the addition of random effects to the model.

For the present study, LMM were used to address the hypotheses of interest. Treatment was included as a fixed factor in each model to explore the difference between WBB and placebo. Baseline performance was also included in each model as a fixed factor covariate to control for pre-treatment individual differences only. Finally, the random effect of participants on intercepts was included in all models to control for individual variation around the by-participant mean. In all models, pairwise comparisons were used where appropriate and Bonferroni corrections were applied to control for type I error.

For all dependent variables, excluding those derived from the MANT, performance was averaged across items for each participant. Therefore, for these tasks it was not possible to include the random effects of items on intercepts. Alternatively, the random effects of items on intercepts were included when analysing the MANT to control for inter-item variability. Further, for MANT alone, Congruency (congruent, incongruent), Load (high load, medium load), and Target Time (120 ms, 500 ms) were additionally included as fixed factors to detect changes in relation to cognitive demand. These were combined with Treatment to assess any interactions with performance (Treatment*Congruency, Treatment*Load, Treatment*Target Time). In addition to the random effect of Participant, for MANT alone the random effect of item or trial was included to control for inter-trial variability; specifically the random effect of trial on intercepts was included to control for individual variation of each trial from the overall group intercept for both MANT accuracy and reaction time after controlling for fixed effects.

All data were normally distributed and there were no extreme outliers beyond of 1.5 standard deviations. Only reaction times for the MANT were excluded if below <100 ms.

2.4 Results

2.4.1 Background measures

The below table (Table 2.4.1) presents the means, standard deviations and ranges of raw scores and standard scores for background measures, as well as key demographic information such as age, gender and school age group. As stated above, independent-

samples t-tests were used to analyse the differences between the demographic information and performance on background measures between treatments.

No significant differences between treatment groups for any of the background measures or demographic information were observed suggesting both treatment groups were evenly matched in terms of: age, gender, school age group, non-verbal intelligence, sustained attention, reading ability. For both treatment groups, RCPM performance was matched to the British Percentile norms whereby a score of 25 was the norm for children aged eight years (the mean age for both treatment groups; Raven, 1981). This highlights that this cohort of children had average non-verbal intelligence levels. When considering the CPT percentage commission errors and percentage omission errors, this cohort achieved slightly higher percentage errors for both outcome measures compared to the norms (commission error mean = 58 – 68% dependent on gender; omission error mean = 6.5 – 6.7% dependent on gender; Conners et al., 2003). However, it is important to note that these norms were for children aged nine to eleven. This was the youngest age range used by the study and no rationale was provided for its selection, or why younger children were not examined. Age effects observed by the study suggests that CPT performance improves with age, with older children having fewer percentage errors than those of a younger age where high percentage errors are observed. Therefore, it would be expected that children below the age of nine to eleven, such as recruited in this thesis would have slightly higher normed percentage errors if following this trajectory within the data. Indeed, marginally higher percentage errors for both outcome measures (commission error mean = 88 – 89%; omission error mean = 10 – 11%) were observed for this cohort of seven to ten-year olds demonstrating potential alignment with hypothetical age norms. Last, both groups performed within one standard deviation above the norm for their age group for all YARC components: reading accuracy, reading rate, reading comprehension, SWRT. This indicated that all children had average to good reading ability.

Table 2.4.1: Data for background measures and demographics including raw score means, standard score means, standard deviations, ranges, *t* statistics, and *p* values.

	Placebo (*n* = 25)			**Blueberry (*n* = 29)**				
	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Age	8.22	1.05	7.04-10.4	8.23	.88	7.03-9.11		.930
Gender (male:female)^	13:12	---	---	12:17	---	---	---	---
School Year (3:4:5)	7:8	---	---	7:10	---	---	---	---
Continuous Performance Task								
Omissions (%)	10.74	7.14	---	11.21	16.03	---	-.159	.874
Commissions (%)	88.48	8.21	---	89.17	15.62	---	-.103	.918
Raven's Coloured Progressive Matrices	26.55	6.10	---	26.78	4.40	---	-.154	.878
York Assessment of Reading for Comprehension								
Reading Accuracy								
Raw scores	14.50	7.93	3-32	17.43	9.14	3-40	---	.327

	Standard scores	103.37	16.96	70-126	107.74	11.27	84-130	-1.076	.287

Table 2.4.1: *continued…*

		M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Reading Rate									
	Raw scores (ms)	286.36	92.69	108-457	293.44	90.07	183-578	---	.507
	Standard scores	107.09	15.72	37-95	109.81	11.73	33-87	-.669	.507
Reading Comprehension									
	Raw scores	11.36	2.88	7-16	11.33	2.13	8-16	---	.287
	Standard scores	111.12	17.81	65-130	115.51	13.82	61-130	-.990	.327
Single Word Reading Task									
	Raw scores	40.25	11.82	14-59	45.18	8.89	21-57	---	.801
	Standard scores	103.91	19.56	70-130	111.44	13.73	88-130	.254	.128

Notes. York Assessment of Reading for Comprehension standard scores (*M* = 100, *SD* = 15). ^ No significant differences were observed between the number of males and females receiving each treatment (males: $\chi^2 = .15$, $p = .700$, females: $\chi^2 = .86$, $p = .350$).

2.4.2 Cognitive measures

2.4.2.1 AVLT

Aligning with the previous research (Whyte & Williams, 2015; Whyte et al., 2016), word recall between A1 and A5 increased at each session. When the distractor list B was presented, word recall declined. Further recalls A6 and A7 (short and long delay) demonstrated decreased word recall in comparison to A5 thought to be caused by retroactive interference of the learning of list B (Figure 2.4.2.1A). Means and standard deviations for all AVLT dependent variables are presented in Table 2.4.2.1A.

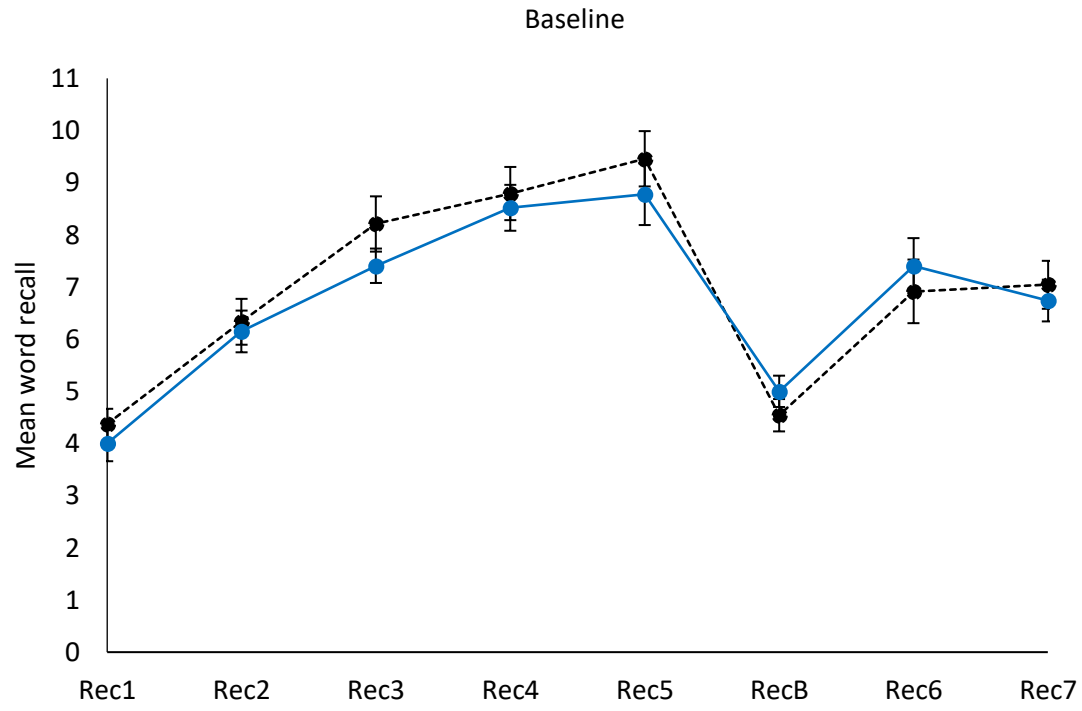
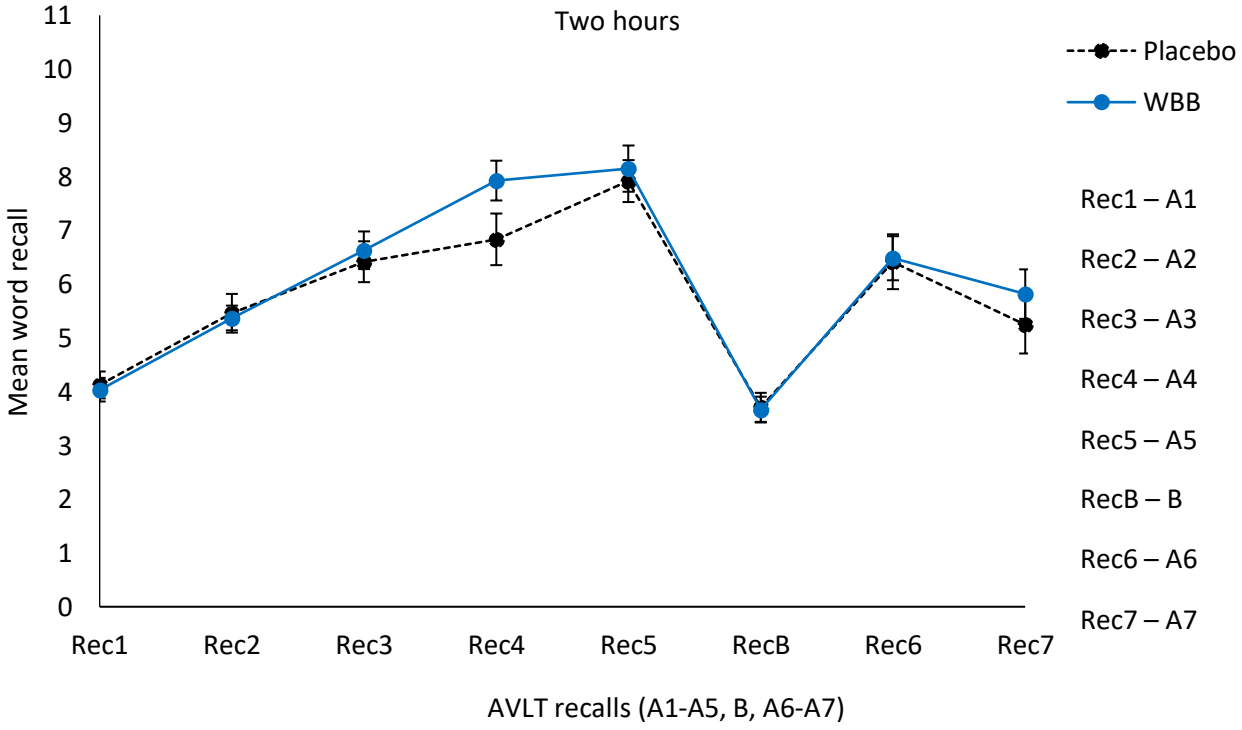


Figure 2.4.2.1A: Mean word recall at each recall point (A1-A5, B, A6-A7) for WBB and



placebo at baseline and two hours post-consumption. Adjusted model means and standard error presented.

Table 2.4.2.1A: Means and standard deviations for all AVLT dependent variables, including *p* values for differences between word recall performance at baseline and two hours post-intervention for each treatment group.

	Baseline					**Two hours**				
	Placebo (n = 25)		**WBB (n = 29)**			**Placebo (n = 25)**		**WBB (n = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Word Span	4.38	1.47	4	1.78	.419	4.13	1.23	4.04	1.19	.769
Words Learnt	5.08	1.99	5.11	3.03	.970	3.79	1.41	4.11	2.04	.525
Final Acquisition	9.46	2.64	9.04	2.58	.567	7.92	1.93	8.15	2.25	.697
Proactive Interference	-.17	1.55	-1	2	.106	.42	1.61	.37	1.21	.908
Retroactive Interference	2.5	2.92	1.33	2.39	.110	1.50	1.98	1.67	1.66	.745
Short Delayed Recall	6.92	3	7.41	2.76	.546	6.08	2.24	6.48	2.16	.521
Long Delayed Recall	5.25	2.69	5.81	2.43	.627	5.25	2.69	6.74	2.10	.435
Total Delayed Recall	13.96	4.8	14.15	4.68	.887	11.33	4.26	12.29	4.22	.422
Total Recall (A1-A5)	37.17	9.83	34.85	8.59	.374	30.75	7.76	32.11	7.31	.522
Word Recognition	10.63	3.45	10.33	3.17	.755	9.21	2.79	9.44	2.03	.729

The fixed effect of Treatment was a significant predictor of total word recall (A1-A5); F(1,51) = .50, MSE = 2.07, p = .035, n^2 = .08 (Figure 2.4.2.1B), with pairwise comparisons revealing significantly greater word recall two hours after WBB compared to placebo (p = .008) where fewer words were recalled.

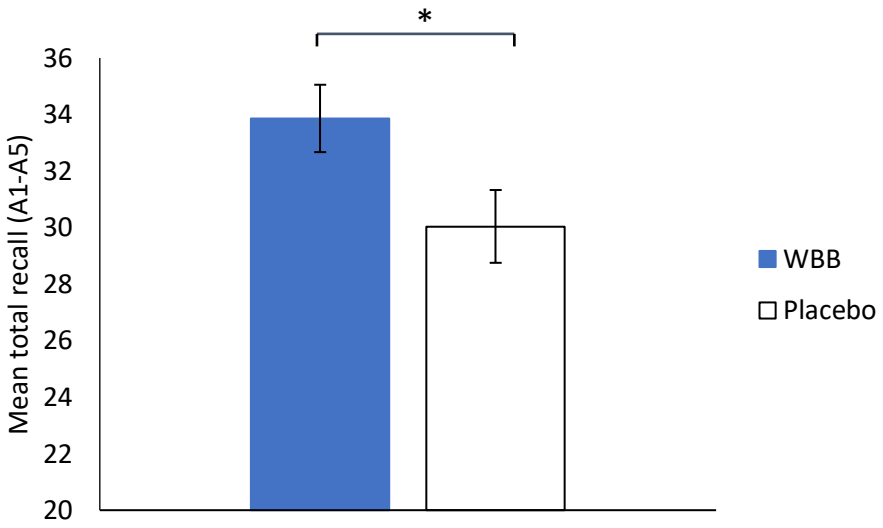


Figure 2.4.2.1B: Significant fixed effect of Treatment for AVLT total word recall (A1-A5; p = .035) with means indicating greater total word recall performance for WBB intake compared to placebo at two hours. Model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.

Treatment was a further significant predictor of short delayed recall performance; F(1,52) = 4.26, MSE = .29, p = .044, n^2 = .05 (Figure 2.4.2.1C), with pairwise comparisons revealing greater word recall two hours after WBB compared to placebo.

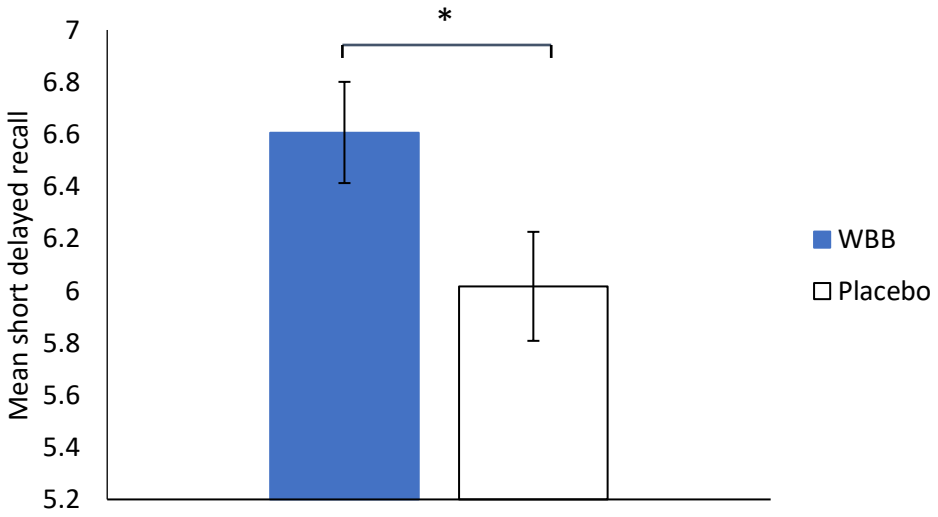


Figure 2.4.2.1C: Significant fixed effect of Treatment for AVLT short delayed recall (p = .044) with means indicating greater short delayed recall for WBB consumption compared to placebo at two hours. Model means and standard error presented, adjusted for baseline performance. LMM with baseline as covariate.

Treatment did not predict performance for any other AVLT dependent variables once Baseline performance was controlled for (Table 2.4.2.1B).

Table 2.4.2.1B: β coefficients and *p* values for the effect of treatment on all AVLT LMM analyses at two hours.

Fixed effects	β coefficients	*p* values
Word Span	.02	.943
Words Learnt	-.56	.224
Final Acquisition	-.48	.320
Proactive Interference	-.01	.986
Retroactive Interference	< -.001	.999
Short Delayed Recall	-.59	.044
Long Delayed Recall	-.82	.164
Total Delayed Recall	-.99	.277
Total Recall (A1-A5)	-3.81	.035
Word Recognition	-.53	.360

2.4.2.2 MANT

2.4.2.2.1 Accuracy

As expected from previous findings (Whyte et al., 2016; 2017), participants in general had greater accuracy for less cognitively demanding trials (congruent, medium load, slow trials), compared to more cognitively demanding trials (incongruent, high load, fast trials) at two hours, regardless of treatment. The fixed effect of Congruency was a significant predictor of accuracy; F(1,404.20) = 20.49, MSE = .02, $p < .001$, $n^2 = .05$, with means indicating greater accuracy on congruent trials compared to incongruent trials. The fixed effect of Target Time further significantly predicted accuracy; F(1,379.37) = 14.37, MSE = .02, $p < .001$, $n^2 = .04$, with means indicating greater accuracy on the slow trials compared to the fast trials. In addition, Load was also a near significant predictor of accuracy; F(1,373.42) = 3.67, MSE = -.03, $p = .056$, $n^2 = .01$, with means revealing greater accuracy on medium load trials

compared to high load trials. In general, accuracy performance was high with participants gaining between 78 – 89% of correct responses at two hours. Means and standard deviations are presented in Table 2.4.2.2.1A.

Table 2.4.2.2.1A: Means and standard deviations for MANT accuracy, including *p* values for differences between accuracy at baseline and two hours post-intervention for each treatment group.

	Baseline					**Two hours**				
	Placebo (n = 25)		**WBB (n = 29)**			**Placebo (n = 25)**		**WBB (n = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency										
Congruent	.83	.15	.83	.13	.929	.89	.16	.86	.15	.533
Incongruent	.67	.19	.68	.19	.827	.77	.18	.77	.17	.999
Load										
High	.74	.15	.74	.16	.878	.81	.16	.78	.15	.780
Medium	.76	.17	.77	.14	.977	.84	.17	.83	.15	.738
Target Time										
120 ms	.72	.18	.72	.17	.893	.79	.19	.79	.17	.797
500 ms	.78	.15	.78	.14	.972	.86	.15	.84	.14	.722

The LMM MANT accuracy analysis revealed that the fixed effect of Treatment was not a significant predictor of accuracy performance when the fixed effects of Congruency, Load, Target Time and Baseline were controlled for, and no significant interactions were observed between Treatment and the MANT parameters (Table 2.4.2.2.1B).

Table 2.4.2.2.1B: β coefficients and p values for all fixed effects and interactions for the MANT accuracy LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
Treatment	.01	.643
Congruency	.03	< .001
Load	-.05	.056
Target Time	-.05	<.001
Treatment*Congruency	.01	.250
Treatment*Load	.01	.976
Treatment*Target Time	-.03	.979

2.4.2.2.2 Reaction time for correct responses only (ms)

The pattern of results for the present data demonstrates that, as expected, participants had faster responses to trials exerting less cognitive demand (congruent, medium load, slow trials) compared to those exerting high cognitive demand (incongruent, high load, fast trials) at two hours post-consumption, regardless of treatment. The fixed effect of Congruency was a significant predictor of reaction time; F(1,398.39) = 77.08, MSE = -51.99, $p < .001$, $n^2 = .16$, with means indicating faster reaction times for congruent trials in comparison to incongruent trials. The fixed effect of Load was a significant predictor of reaction time; F(1,369.71) = 4.16, $p = .042$, $n^2 = .01$, with means indicating faster reaction times for medium load trials in comparison to high load trials. Target Time did not significantly predict reaction time. Means and standard deviations are presented in Table 2.4.2.2.2A.

	Baseline					Two hours				
	Placebo (n = 25)		WBB (n = 29)			Placebo (n = 25)		WBB (n = 29)		
	M	***SD***	***M***	***SD***	***p values***	***M***	***SD***	***M***	***SD***	***p values***
Congruency										
Congruent	549.79	89.75	529.21	113.63	.469	544.84	65.88	513.08	116.79	.234
Incongruent	621.36	122.26	585.81	155.91	.361	618.15	83.04	579.07	141.42	.231
Load										
High	594.09	106.50	565.48	138.11	.404	588.59	78.58	554.82	133.27	.272
Medium	577.06	101.79	548.69	129.39	.380	554.82	72.21	537.35	123.21	.193
Target Time										
120 ms	550.24	118.09	530.94	140.81	.591	573.69	77.02	529.55	137.25	.160

500 ms	620.91	100.92	583.04	129.70	.242	589.30	86.90	562.46	121.92	.363

Table 2.4.2.2.2A: Means and standard deviations for MANT reaction times (ms; correct responses only), including *p* values for differences between reaction times at baseline and two hours post-intervention for each treatment group.

A significant Treatment*Target Time interaction was apparent when all fixed effects were controlled for; F(1,367.80) = 5.64, MSE = 46.01, p = .018, n^2 = .88. Pairwise comparisons revealed a trend between treatments for 120 ms trials (p = .078) where those receiving WBB had faster reaction times compared to those receiving placebo, as shown in Figure 2.4.2.2.2. Although not significant, this indicates that acute WBB intake has the potential to positively benefit reaction time performance for trials requiring high cognitive demand.

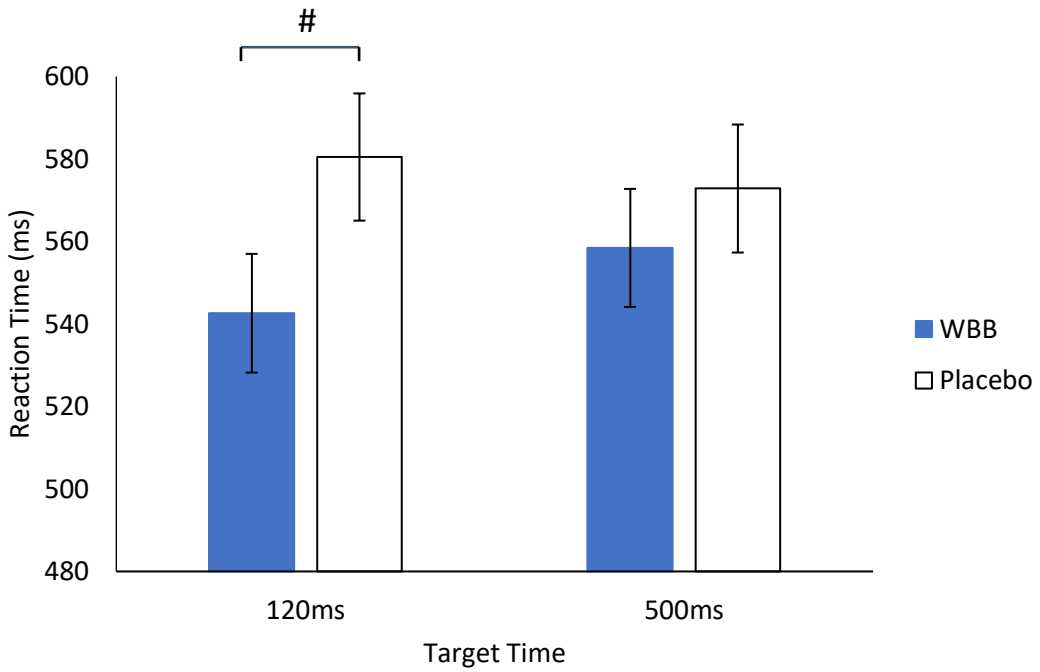


Figure 2.4.2.2.2: The significant Treatment*Target Time interaction for MANT reaction time (p = .018) with a near significant difference between treatments for 120 ms trials (p = .078), suggesting faster reaction times at two hours after WBB consumption. Adjusted model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.

There was no significant effect of Treatment or interactions with the other MANT parameters.

Table 2.4.2.2.2B: β coefficients and p values for fixed effects in the MANT RT LMM analysis (ms; correct responses only) at two hours.

Fixed effects	**β coefficient**	***p* value**
Treatment	6.89	.208
Congruency	-51.99	< .001
Load	5.36	.042
Target Time	-23.71	.456
Treatment*Congruency	.59	.803
Treatment*Load	12.44	.749
Treatment*Target Time	46.01	.018

2.4.2.3 PANAS-C

In general, the pattern of data suggests that at two hours, participants had greater feelings of positive affect but lower feelings of negative affect, regardless of treatment. Means and standard deviations are presented in Table 2.4.2.3A.

Table 2.4.2.3A: Means and standard deviations for positive affect and negative affect, including *p* values for differences between mood at baseline and two hours post-intervention for each treatment group.

	Baseline					**Two hours**				
	Placebo (n = 25)		**WBB (n = 29)**			**Placebo (n = 25)**		**WBB (n = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Positive	49.21	10.04	49	11.96	.116	48.29	11.89	52.36	9.67	.947
Negative	19.25	3.86	20.79	7.43	.685	19.79	5.48	20.07	7.71	.366

When assessed independently, the fixed effect of Treatment significantly predicted positive affect; F(1,52) = 4.98, MSE = -4.22, p = .030, n^2 = .09 (Figure 2.4.2.3). Here, pairwise comparisons revealed that those receiving WBB had greater self-reported positive affect scores compared to those receiving placebo at two hours (p = .027; Table 2.4.2.3B).

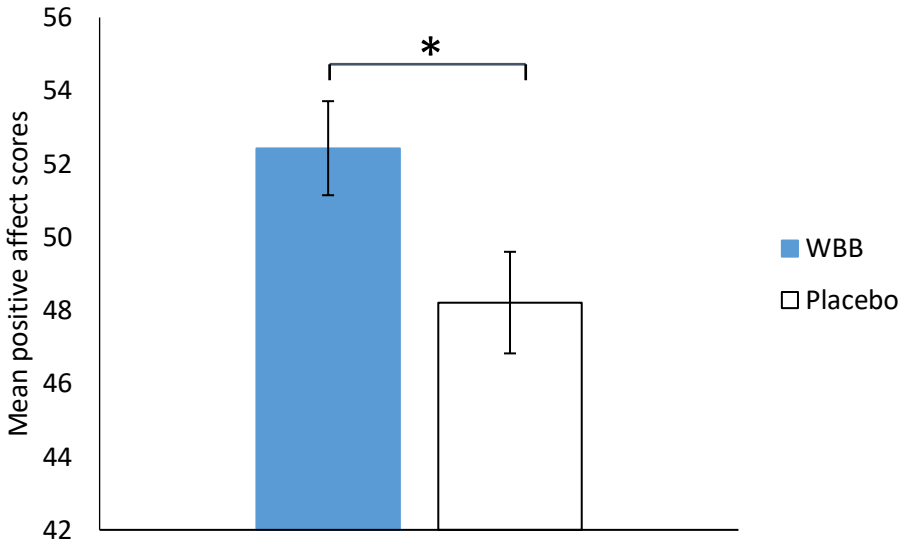


Figure 2.4.2.3: Significant fixed effect of Treatment for positive affect (p = .030) whereby those receiving WBB had greater self-reported positive affect at two hours compared to placebo. Adjusted model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.

Treatment was not a significant predictor of negative affect (Table 2.4.2.3B).

Table 2.4.2.3B: β coefficients and p values for the fixed effect of Treatment for the LMM PANAS-C analyses at two hours.

	Fixed effects	**β coefficient**	***p* value**
Positive Affect		-4.22	.030
Negative Affect		.51	.761

2.4.2.4 TOWRE-2

In general, TOWRE-2 performance was greater for SWE compared to PDE whereby participants read aloud more words correctly than non-words at two hours, regardless of treatment. This was expected and demonstrates that participants read aloud more words that they already knew within the 45 seconds compared to non-words they had no prior knowledge of. Therefore, fewer non-words were read aloud as participants had to spend more time decoding and learning the new words. Means and standard deviations are presented in Table 2.4.2.4A.

Table 2.4.2.4A: Means and standard deviations for SWE and PDE at baseline and two hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 25)		**WBB (n = 29)**			**Placebo (n = 25)**		**WBB (n = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
SWE	60.67	13.06	64.74	12.18	.255	61.42	13.63	65.70	12.06	.239
PDE	30.46	12.37	33.93	9.52	.265	31.58	12.99	34.48	9.99	.373

The fixed effect of Treatment did not significantly predict either SWE performance or PDE performance demonstrating no effect of treatment on reading behaviour (Table 2.4.2.4B).

Table 2.4.2.4B: β coefficients and p values for the fixed effect of Treatment for TOWRE-2 SWE and PDE LMM analyses at two hours.

	Fixed effects	**β coefficient**	***p* value**
SWE		-.46	.739
PDE		.46	.713

2.5 Discussion

The present study aimed to examine the effects of acute WBB supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds. Consumption of the WBB treatment was found to significantly improve total word list recall with greater recall observed two hours after treatment, in comparison to placebo. Additionally, WBB intake significantly improved short delayed recall performance with a greater number of words recalled after a two-minute delay, two hours after treatment in comparison to placebo. When analysing the executive function data, a significant Treatment x Target Time interaction was apparent, with pairwise comparisons revealing a trend for faster reaction times on the more cognitively demanding 120 ms trials following WBB intake compared to placebo. Further, those consuming WBB reported significantly greater feelings of positive affect two hours after treatment, in comparison to placebo. This suggests that the acute WBB treatment helped boost positive mood over the two-hour period; no effect of WBB supplementation was observed for feelings of negative mood. Last, although participants showed greater fluency and efficiency for the reading of words compared to the reading of non-words, no difference was observed between treatments for words or non-words, suggesting that on this occasion, acute WBB supplementation did not affect reading behaviour.

As originally hypothesised, the present study aimed to extend previous findings from Whyte et al. (2016). A number of similarities in significant findings between the two studies were observed which collectively demonstrate that a single, one-off dose of a flavonoid rich WBB treatment can improve both word list recall and executive function. However, subtle differences were still apparent requiring further explanation and exploration. In line with Whyte et al. (2016), the present study observed significant improvements to word list recall and short delayed word recall two hours after WBB. This was in comparison to Whyte et al. (2016) who only observed a trend for improved delayed word recall six hours after the WBB treatment. However, not all word recall and recognition findings from Whyte et al.'s research were observed in the present study; for example, no effects of acute WBB consumption were observed for word recognition; an attenuation of word recognition performance from Whyte et al. (2016) was originally observed up to six hours post-consumption of the 30 g WBB treatment.

One explanation for the present study failing to observe an acute WBB effect for word recognition is that such an effect may only be observed at a later test session following treatment consumption; indeed the present study only examined performance two hours after treatment, unlike Whyte et al. (2016) who observed this effect up to six hours later. These studies suggest that acute WBB may affect different word list recall components of episodic memory at different post-prandial time points; for example, word list recall shows immediate effects one to two hours after treatment for both studies, whilst word retrieval as measured by word recognition shows delayed effects up to six hours after treatment (Whyte et al., 2016). This may further reflect a difference between how WBB supplementation acts upon stimulus encoding, as portrayed in this instance by word list recall, and stimulus retrieval as portrayed by word recognition. This is one future avenue for research to explore. Of greater relevance to the present research hypothesis, the two sets of data begin to demonstrate a general pattern of results highlighting the aspects of episodic memory that are improved following acute WBB treatment, and when these improvements occur after treatment.

In addition to episodic memory improvements for total word list recall and short delay word recall, the present study observed a trend for improvements to executive function reaction times, particularly for more cognitively demanding 120 ms trials two hours after WBB intake. This is again similar to that found by Whyte et al. (2016), who observed improvements to executive function accuracy on the ANT three hours after WBB supplementation, specifically on trials requiring greater cognitive demand. Therefore, the executive function results from the present study further demonstrate the potential for acute WBB supplementation to improve executive function performance at two hours after WBB intake. However, the present finding was specific to MANT reaction time and not MANT accuracy as found by Whyte et al. (2016). Instead, this finding mirrors Whyte et al.'s (2017) later research which observed improvements to executive function reaction times for more cognitively demanding trials on the MANT. One explanation for the absence of acute WBB effects for executive function accuracy is that all children, regardless of treatment group, were forming at ceiling for this task, scoring between 78 – 89% correct responses at two hours. Therefore, it may be unrealistic to expect an improvement to performance when children are already showing highly accurate performance.

Further, the present finding was only apparent for more cognitively demanding fast Target Time trials (120 ms) and not for the other MANT components: Congruency and Load. It is also important to note that this was the only interaction (Treatment x Target Time) to reach significance in the present study when analysing the executive function data, and when explored further only a trend towards significance was observed between WBB and placebo for these trials. In this instance, it is vital to consider what is driving this interaction, such as the effect of type 1 error when completing multiple comparisons, although Bonferroni corrections were applied. This is of particular relevance as when the fixed effects of Treatment and Target Time were analysed independently, no significant differences were detected between treatments or the two target times: 120 ms and 500 ms. Overall, this finding from the present study indicates the potential of acute WBB supplementation to improve sustained attention, inducing faster reaction times for fast trials (120 ms) requiring greater cognitive demand. However, further research is needed to examine the effect of acute WBB supplementation on executive function performance to provide further clarification of which executive function outcome measure (accuracy, reaction times) is most susceptible to treatment, and whether these improvements are greater for more cognitively demanding trials (incongruent, high load, 120 ms trials) which span across multiple MANT components (congruency, load, target time).

One explanation for the subtle differences observed between the two studies, both of which employed the same cognitive tests and treatments, highlights the possibility that the time when participants were tested may have had an impact on the results obtained. The time of day when cognitive testing took place differed between the present study and Whyte et al. (2016). In the present study, both test sessions (baseline and post-consumption) and treatment consumption were completed in the afternoon after lunch to fit in around the school day, minimising disruption to classes. However, testing commenced throughout the whole school day for Whyte et al. (2016); baseline testing (0830 hours), treatment consumption (0900 hours), and an initial post-consumption test session at 75 minutes after treatment consumption (1015 hours) all occurred in the morning. Subsequent test sessions at three hours (1200 hours) and six hours (1515 hours) were then completed in the afternoon. Therefore, the present study's findings from test sessions assessing performance in the afternoon (baseline and post-consumption) realistically map onto Whyte

et al.'s afternoon test sessions (three hours and six hours), not the baseline testing and the initial post-consumption test session (75 minutes) which were completed in the morning.

In this instance, it is plausible that time of day may have influenced performance, and that the effects of WBB consumption on performance may differ when consumed in the morning compared to the afternoon. For example, significant word list recall was observed at 75 minutes after acute WBB intake when consumed in the morning by Whyte et al. (2016), however these word list recall improvements did not extend to the later test sessions at three hours and six hours (in the afternoon post lunch). Alternatively, the present study observed significant improvements to both total recall and short delayed recall two after WBB supplementation when testing took place in the afternoon. Further, Whyte et al. (2016) observed an attenuation in the natural decline of word recognition performance up to six hours following WBB consumption. No effect of acute WBB consumption on word recognition was observed for the present study. Last, Whyte et al. (2016) observed significant improvements to executive function accuracy in the afternoon at three hours after WBB supplementation, a finding mirrored by the present study but for executive function reaction times two hours after WBB supplementation when assessed in the afternoon. Therefore, the AVLT and ANT/MANT tasks utilised by both studies are producing different results depending on when the WBB treatment was given and when cognitive testing took place. As of yet, it is unclear from the two data sets whether the time of day (morning or afternoon) is indeed having a potential effect on the cognitive outcomes as, thus far, studies examining the effect of acute WBB treatments on children's cognition are limited. Therefore, it would be important for future research to consider the implication of time of day and its potential to alter the effects of WBB supplementation on children's cognitive performance (Chapter 3).

In general, when performance was assessed in the afternoon, both studies showed that those receiving placebo had worse performance or showed a continuing decline in performance. This was in comparison to those receiving WBB where either an improvement or an attenuation in performance was observed. Specifically, the present study demonstrated that acute WBB supplementation has the potential to improve to executive function reaction times for more cognitively demanding 120 ms trials in comparison to placebo where performance was significantly worse two hours after treatment. This was in addition to an improvement to total word recall and delayed word recall two hours after

WBB supplementation, again in comparison to placebo where a decline in performance was observed two hours later. In parallel, Whyte et al. (2016) observed an attenuation in word recognition and delayed word recall performance three and six hours after WBB intake, compared to placebo where performance on these episodic memory measures continued to decline. Collectively, these results suggest that performance when examined in the afternoon gradually declines over time, but that acute WBB consumption has the potential to attenuate, or even improve, performance at this time of day.

One explanation is that WBB supplementation may exert benefits to cognition when children experience cognitive fatigue. In the afternoon, children may perform below their cognitive optimum due to experiencing fatigue from continuous learning throughout the school day. The influence of processing and learning school material may cause proactive interference and negatively affect the processing and completion of the cognitive measures. To this extent, not only did the present study show that acute WBB consumption has the potential to benefit performance when tested in the afternoon, but when performance is influenced by cognitive fatigue, causing a greater demand on cognitive resources. This is in line with the significant improvements observed for executive function performance for more cognitively demanding trials observed both in this chapter and by Whyte et al. (2017). Further, this supports and builds upon Whyte et al.'s (2016) findings whereby the natural decline in word recognition and delayed word recall observed for placebo was attenuated following WBB consumption for the afternoon test sessions. However, it is important to note that subjective feelings of fatigue were not measured by this study. Future research should include a measure of fatigue at baseline and post-treatment to assess the potential effects of treatment of subjective fatigue, in particular when testing children in the afternoon when cognitive fatigue is thought to be apparent.

In addition to cognitive fatigue and proactive interference, lunch consumption prior to testing could influence performance by causing the general decline in cognitive performance observed by those receiving placebo. Research in adults indicates that lunch consumption negatively impacts upon cognitive performance post-prandially (Smith & Miles, 1986a); referred to as the post-lunch dip. However, the limited research conducted thus far in children demonstrates no effects of post-lunch dip on cognition, executive function in particular (Schröder et al., 2015; 2016). The absence of a pre-lunch test point means that a possible post-lunch dip effect remains inconclusive and requires further investigation in

children. In order to specifically examine the effects of WBB on overcoming the post-lunch dip in children, future research may wish to consider testing performance before and after lunch itself. It is also important to consider that the cognitive benefits seen for WBB intake, in comparison to placebo, could be influenced by the lunch itself. In this instance, the lunch time meal was not standardised due to children having the choice of either a packed lunch or school dinner, although flavonoid intake was restricted as participants followed a 24-hour low-flavonoid diet whilst completing the study. For practical reasons in the school environment the content of lunch could not be assessed, however there is no reason to believe that there were significant differences in macronutrient intake between the treatment groups. Further, lunch did not occur at a standardised point prior to baseline testing. The inconsistencies in time between lunch consumption and baseline testing could have further influenced performance, however due to the regimented testing timetable and the lunch period occurring for a set duration only, it was not possible for all participants to have a standardised lunch time. Therefore, the effect of lunch on cognitive performance cannot be ruled out in this instance, although the present findings suggest that acute WBB supplementation can maintain and improve performance on certain cognitive tasks of episodic memory and executive function. It would be ideal for future research to consider the inclusion of a standardised lunch consumed at a set time before testing takes place.

Further to the cognitive benefits following acute WBB supplementation, benefits to children's mood were seen. This is the first time research has explored the effects of acute WBB intake on mood in children; not measured previously by Whyte and Williams (2015) or Whyte et al. (2016; 2017). Here, significant improvements in self-reported positive affect were apparent two hours after WBB consumption; no effects were observed for negative affect. This finding builds upon the limited berry-flavonoid and mood literature in adults which positively associates berry-flavonoid consumption with mood improvements (Khalid et al., 2017; Watson et al., 2019). However, despite the recent interest in berry-flavonoids and mood, the underlying mechanisms of action are currently unknown, although a number of potential mechanisms may underlie this effect: increased CBF, monoamine oxidase, GABA receptors (Section 1.1.1.5). As an emerging field of interest, future research should consider employing a standardised measure of mood and study design in order to reliably establish the positive benefits of berry-flavonoids on mood.

Despite the significant improvements to both cognition and mood following acute WBB consumption in the present study, no effects of acute WBB supplementation were seen on reading behaviour for either known words or non-words. As mentioned in Section 2.1, phonology, orthography, and semantics, are relied upon for efficient word reading and must function simultaneously to achieve this goal. These skills develop alongside executive functions such as working memory, inhibition, and sustained attention, which in turn enhances the process of learning to read words (Cartwright, 2012). Therefore, due to the demand placed on these multiple resources to function effectively together, it is plausible that a one-off dose of WBB may not be sufficient to elicit changes to reading as a whole. This is in comparison to the cognitive benefits seen here where aspects of cognition that reading relies upon were assessed independently. Future research should consider the impact of sustained WBB supplementation on reading behaviour, such as a daily dose given chronically, to examine whether prolonged berry-flavonoid supplementation is more beneficial to the complexity of cognitive domains that underpin reading compared to a single, one-off dose.

Further, the majority of children from this sample were good readers: for YARC accuracy 75% performed above the standard score mean (*M* = 100; Snowling et al., 2009), for YARC reaction time 80% performed above the standard score mean, and for YARC comprehension 91% performed above the standard score mean. Therefore, it is possible that these results cannot be generalised to children who perform below the standard mean, an indicator of poorer reading ability. It would be interesting for future research to examine the effect of acute WBB supplementation on children with atypical reading performance, such as those who perform below expectations for their age. This is consistent with the previous research examining the effects of PUFAs supplementation on reading in children where significant improvements were only apparent in children with learning/behavioural difficulties or underperforming individuals (Richardson and Montgomery, 2005; Richardson et al., 2012).

In addition, the TOWRE-2 may not be sensitive enough to detect changes to reading behaviour following acute WBB consumption; as of yet, this measure has not been used to assess the effect of dietary interventions on reading behaviour. The TOWRE-2 measures the fluency and accuracy of known and novel orthography when read aloud within a short period of time (45 seconds). Essentially, this timed element measures participants' automaticity of reading aloud prior letter-sound correspondences and, in particular, may

detract participants from making a correct response on the phonemic decoding subtest. Instead of focusing on making a correct decoding effort, children focus on reading aloud as many non-words as possible within the time. To account for this potential lack of sensitivity, future research should focus on a select and more specific aspect of learning to read, without a time limit. For example, research could examine how children learn to read words which focuses on learning the mappings between orthography (a written word) and phonology (pronunciation). This is how children learn to read naturally and would allow the examination of how WBB intake impacts upon a developmental aspect of reading outside of a research setting.

Before further investigation into the effects of acute WBB supplementation on reading behaviour, it is first important to understand why subtle differences in episodic memory and executive function are apparent between the present study and Whyte et al. (2016). As these are the first studies to examine the role of acute WBB consumption on the cognition of children, it is vital to follow up these initial findings to establish consistency, and to consider time of day as a factor potentially responsible for these differences.

To conclude, acute WBB supplementation demonstrated a positive benefit to the cognition and mood of seven to ten-year olds. Here, greater word list recall for total acquisition and short delayed recall was observed two hours after WBB intake, in comparison to placebo. Further, acute WBB supplementation may have the potential to improve executive function reaction times for trials requiring greater cognitive demand, specifically for faster trials (120 ms). This study set out to extend initial findings from Whyte et al. (2016), however differing methodologies, specifically the time of day when children were tested, are believed to have led to subtle differences in the results obtained. As stated above, it is important to understand why these differences in results have occurred, and to fully understand the role of acute WBB supplementation on children's cognition before further exploration on reading behaviour can occur. The novel benefit of acute WBB intake for improving self-reported positive affect adds to the emerging literature investigating the potential of berry-flavonoids to improve mood. However, these positive benefits did not extent to reading behaviour, with no improvements observed for the reading aloud of words or non-words in this instance. Nevertheless, the data highlights that acute WBB supplementation can improve the cognitive aspects that underlie reading, such as word learning via list recall, thus suggesting that improvements to reading behaviour in children

are not unrealistic to expect. In this case, the duration of WBB supplementation may have hindered the potential of the WBB treatment to improve reading behaviour so it is now important to advance this research by examining the effect of chronic WBB supplementation on reading.

Following on from the present study, Chapter 3 will examine whether time of day impacts the effect of acute WBB supplementation on cognition, reading behaviour, and mood, in the same target population of seven to ten-year olds. This study aims to support the present acute study by testing children in the morning. Data collected from the present study where children were tested in the afternoon will then be compared directly to the data collected in Chapter 3 to examine whether the effects of acute WBB supplementation on cognition, reading behaviour, and mood, differ as a function of time of day.

Chapter 3

Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds – an interaction with time of day

3.1 Introduction

The previous chapter aimed to extend research by Whyte et al. (2016) which observed significant improvements to word list recall as measured by the AVLT, and executive function accuracy on the more cognitively demanding MANT trials after consumption of an acute 30 g freeze-dried WBB powder treatment. In addition, maintenance of delayed word recall and word recognition performance were further observed up to six hours after intake of both a 15 g and the 30 g freeze-dried WBB powder treatments compared to placebo. The first study (Chapter 2) examined the acute effects of the same 30 g freeze-dried WBB powder treatment on the cognition, reading behaviour, and mood, of seven to ten-year olds. Significant improvements were observed for total recall and short delayed recall with a greater number of words recalled for both measures two hours after acute WBB consumption in comparison to placebo. Further, a trend towards improved executive function reaction time was observed with faster responses for the more cognitively demanding trials (120 ms fast trials) two hours after WBB supplementation, compared to placebo and the less cognitively demanding trials (500 ms slow trials). Last, a significant improvement to self-reported positive affect was observed two hours after WBB supplementation compared to placebo. However, contrary to the present hypotheses acute WBB treatment did not affect reading behaviour for either sight word efficiency or phonemic decoding efficiency.

Despite the positive benefits to cognition following acute WBB supplementation, subtle differences were apparent between the two studies, potentially caused by differences in the methodologies used. For example, the time of day when participants were tested and when treatment was administered differed between the two studies. As discussed in Section 2.5, Whyte et al. (2016) observed a general decline in word list recall performance at the three and six-hour test sessions compared to baseline. However despite this decline, consumption of both the 15 g and 30 g WBB treatments was shown to attenuate the decrease in word recognition performance at these afternoon test sessions, whilst a trend was observed for the intake of the 15 g treatment to further attenuate delayed word recall decline at these

later test sessions. In contrast, Study 1 found significant total word recall improvements when performance was assessed in the afternoon two hours after consumption of the 30 g WBB treatment, whilst Whyte et al. (2016) only observed word acquisition improvements in the morning 75 minutes after WBB consumption. Next, Whyte et al. (2016) observed significant improvements to executive function accuracy in the afternoon three hours after consumption of the 30 g WBB treatment. Although Study 1 also observed a significant improvement to executive function performance two hours after intake of the 30 g treatment when assessed in the afternoon, this improvement was for reaction time and not accuracy.

These differences in findings suggest that time of day may interact with the effect of acute WBB supplementation on cognitive performance. In general, cognitive functioning fluctuates over the 24-hour period and is strongly associated with alertness levels (Åkerstedt et al., 2008). Greater alertness is generally experienced in the morning when sleep pressure is at its lowest; although sleepiness is still exhibited first thing in the morning as a result of sleep inertia (a subjective feeling of grogginess after waking) which can reduce cognitive performance initially in the early morning. However sleep inertia, and therefore a lack of alertness, dissipates approximately one hour after waking (Jewett et al., 1999). As sleep pressure builds up throughout the day towards bedtime, alertness decreases; a decrease is also exhibited in the early afternoon, independent of food intake (Bes et al., 2009). Subsequently, as alertness decreases, cognitive performance is negatively affected and decreases in turn. Research by Janvier and Testu (2007) found that ten to eleven-year olds showed lowest performance levels on a visual discrimination task in the early morning (0850 hours) and early afternoon (1350 hours), with highest levels found in late morning (1120 hours). Further, Cerasuolo et al. (2016) observed an association between increases in self-reported sleepiness and poor executive function performance in five to eleven-year olds. As ratings of sleepiness increased from the morning (0800 – 1030 hour), to mid-day (1100 – 1300 hour) and into the afternoon (1400 – 1600 hour), executive function accuracy decreased with worse performance in the afternoon compared to mid-day, and worse performance at mid-day compared to the morning. This pattern of cognitive fluctuations throughout the day is supported by the previous acute WBB research in children (Whyte et al., 2016). Specifically, the control group recruited by Whyte et al. (2016) demonstrated greater word recall in the morning, whilst word recall performance gradually decreased

throughout the afternoon test sessions. This pattern was further observed in Study 1 where the control group exhibited a decline in word recall and executive function performance when tested in the afternoon.

3.1.1 The present study

The present study aimed to examine whether time of day interacts with the effect of acute WBB supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds. Therefore, Study 2 set out to examine how the effects of acute WBB supplementation differ for morning and afternoon performance by testing for a potential time of day by treatment interaction for cognition, reading behaviour, and mood. To achieve this, data collected in the morning (Study 2) and data collected in the afternoon (Study 1) from different cohorts of children were combined to assess this potential interaction. In order to replicate the previous afternoon study (Study 1) and to allow for this direct comparison between results, a between groups, randomised, single-blind, placebo-controlled study design was applied within a new cohort of children. In line with previous literature from children (Study 1; Whyte & Williams, 2015; Whyte et al., 2016; 2017), a target population of seven to ten-year olds were recruited and randomised to receive either the 30 g freeze-dried WBB powder treatment or placebo. Participants completed the same tasks as in Study 1 for word list recall and recognition, executive function, reading behaviour, and mood, tested at baseline and two hours after treatment. Data collected in the morning was then compared to data collected in the afternoon (Study 1).

In general, the present study hypothesised that the 30 g freeze-dried WBB powder treatment given in the morning would improve total word recall and short delay word recall, executive function, and positive affect two hours after treatment consumption compared to the placebo; to align with the previous findings (Study 1). Secondly, it was hypothesised that acute WBB given in the morning would aid performance on the more cognitively demanding executive function trials, in comparison to the less cognitively demanding trials and compared to a placebo, for both executive function accuracy and reaction time. Next, it was hypothesised that acute WBB treatment given in the morning would have a positive effect on reading behaviour two hours after treatment consumption, compared to a placebo. Although the previous study (Study 1) did not observe an effect of acute WBB intake on either sight word efficiency or phonemic decoding efficiency, it is possible that reading

behaviour may be more sensitive at different times of day, such as when alertness levels are greater in the morning. Therefore, acute WBB supplementation may have the potential to improve on reading behaviours in the morning.

To align with the previous research on cognitive fluctuations throughout the day (Åkerstedt et al., 2008), it was hypothesised that greater performance would be observed in the morning for word list recall and recognition, executive function, reading behaviour, and mood, regardless of treatment, as it is at this time of day when alertness levels and subsequently cognitive function performance are greater in children (Janvier & Testu, 2007; Cerasuolo et al. 2016).

When considering the potential time of day by treatment interaction, it was hypothesised that time of day would interact with the effects of acute WBB supplementation on performance. In light of the evidence above, greater performance for word list recall, executive function, reading behaviour, and mood, was expected in the morning compared to afternoon following WBB compared to placebo.

3.2 Methods

The present study (for ethical approval, see Appendix A.1) replicated the methodology used previously in Study 1 (Section 2.2) with only time of day, when treatment was administered and when participants were tested, differing between studies. In the present study all participants underwent baseline testing, treatment administration, and two-hour post-intervention testing in the morning, whilst these stages all took place in the afternoon for the previous study.

3.2.1 Participants

An *a priori* power analysis (using G Power 3.1) based on the significant AVLT total word recall finding from Study 1 was used to calculate the required sample size. A two-tailed test with a partial alpha of $p = .05$ revealed a total of 50 children would be required to achieve sufficient power of 0.8 ($n = 21$ per treatment group) when employing a between-subjects design.

A total of 61 healthy children (31 males: 30 females) aged seven to ten years ($M = 8.20$, $SD = .87$) were recruited from two primary schools local to the University of Reading. Written consent was obtained from parents/legal guardians (see Appendices B.1 & C.1) prior

to participation, as well as participant's assent (see Appendices D.1 & E). Parents/legal guardians confirmed their child spoke English as a first language, had no known learning or behavioural difficulties (e.g. ADHD, dyslexia or reading impairments), and had no fruit or fruit juice intolerance. All 61 participants completed the study in full.

3.2.2 Background measures

Participants completed a series of background measures during an initial practise session: Raven's Coloured Progressive Matrices (RCPM; Raven, 1965), Continuous Performance Task (CPT; Conners, 1994), York Assessment of Reading for Comprehension (YARC; Snowling et al, 2009; see Appendix G), and the Single Word Reading Task (SWRT; see Appendix H) sub-test of the YARC. See Section 2.2.2 for further information on the cognitive domains and reading components assessed by these background measures. Raw scores and standard scores for these tasks are shown in Table 3.4.1.

3.2.3 Treatments

Participants were randomly allocated to receive either the 30 g freeze-dried WBB powder treatment or the sugar-matched placebo (*n* = 31 WBB, *n* = 30 placebo). See Section 2.2.3 for further information on treatment constituents, preparation and administration.

3.2.4 Cognitive measures

As in Study 1, the measures of cognition, reading behaviour, and mood, were: Rey's Auditory Verbal Learning Task (AVLT; Lezak, 1983; see Appendix I), Modified Attention Network Task (MANT; Fan et al., 2002, Whyte et al., 2017), Test of Word Reading Efficiency (TOWRE-2; Torgesen et al., 2012; see Appendix J), and the Positive and Negative Affect Scale for Children (PANAS-C; Laurent et al., 1999; see Appendix K). See Section 2.2.4 for further information on the cognitive domains, reading behaviours, and mood affects assessed by each task.

3.2.5 Procedure

3.2.5.1 Practise Session

Consistent with Study 1, participants took part in an initial practise session one to two days prior to the main test day. Here, participants completed the background measures as well as a practise of the AVLT and MANT tasks in order to eliminate practise effects. After,

participants followed a 24-hour low-flavonoid diet (see Appendix F) prior to the main test day.

3.2.5.2 Test Day

Participants were tested individually in a quiet space at their schools. After following a low-flavonoid breakfast as a part of the 24-hour low-flavonoid diet, participants completed the baseline session at either 0900 hours, 0930 hours, or 1000 hours immediately before treatment or placebo administration. After consumption, participants returned to class for a two-hour period where they were only allowed to consume water and abstained from exercise. Cognitive performance was assessed two hours after treatment at either 1140 hours, 1210 hours, or 1240 hours (dependent on time of consumption). All participants were asked to abstain from eating lunch until after their post-treatment test session. This was to avoid the potential interference of lunch on the treatment and to rule out any effects of lunch on cognitive performance (Section 2.5). Testing concluded after this second test session. See Section 2.2.5 for further information on the practise session and test day procedures.

3.3 Statistical analysis

Data were analysed using SPSS (Version 21.0). For participants tested in the morning, differences between treatment groups (WBB, placebo) for background variables: Raven's non-verbal IQ, CPT omissions and commissions, general reading ability, and SWRT, were examined using independent-samples t-tests. A chi-square was performed to assess sex differences between the treatment groups. Means, standard deviations and group comparisons for data collected in the morning are presented in Table 3.4.1. Means, standard deviations and group comparisons for data collected in the afternoon are presented in Table 2.4.1. For the combined data set, a 2 x 2 (Time of Day*Treatment) ANOVA was used to analyse the differences between the background measures and demographic information at each time of day for each treatment group.

Linear Mixed-effect Models (LMM) using unstructured covariance matrices were used to analyse a combined data set containing data collected in the afternoon (Study 1) and data collected in the morning (Study 2) to explore the potential interaction between time of day and treatment on cognition, reading behaviour, and mood. A separate model was analysed for each dependent variable: all AVLT outcomes measures (see Section 2.2.4.1), MANT

accuracy and reaction time (ms; correct responses only), both TOWRE-2 SWE and TOWRE-2 PDE, and both PANAS-C PA and PANAS-C NA.

For the present study, LMM was used to address the hypotheses of interest. For all models, Treatment was included as a fixed factor to explore the difference between WBB and placebo. Time of Day was included in all models as a fixed factor to explore the difference between morning and afternoon testing. Interactions between Time of Day and Treatment were examined for all outcome measures to assess whether performance differed between time of day for the treatments or between treatments for each time of day. Baseline performance was also included in each model as a fixed factor covariate to control for pre-treatment individual differences only. Random effects of participants on intercepts was included in all models to control for individual variation around the by-participant mean.

For all dependent variables, excluding those derived from the MANT, performance was averaged across items for each participant. Therefore, for these tasks it was not possible to include the random effects of items on intercepts. Alternatively, the random effects of items on intercepts was included when analysing the MANT to control for inter-item variability. Further, for MANT alone, Congruency (congruent, incongruent), Load (high load, medium load), and Target Time (120 ms, 500 ms) were additionally included as fixed factors to detect changes in relation to cognitive demand. These were combined with Treatment to assess any interactions with performance (Treatment*Congruency, Treatment*Load, Treatment*Target Time), as well as combined with Time of Day to assess interactions with performance (Time of Day*Congruency, Time of Day*Load, Time of Day*Target Time). Three-way interactions between Treatment, Time of Day and each MANT parameter were also included to examine the effects of both Time of Day and Treatment on MANT performance for Congruency, Load, and Target Time. In all models, pairwise comparisons were used where appropriate and Bonferroni corrections were applied to control for Type 1 error.

3.4 Results

3.4.1 Background measures

The below table (Table 3.4.1) describes the means, standard deviations, and ranges of raw scores and standard scores for the background measures and key demographic

information such as age, gender, and school age group, for the present data collected in the morning. The same demographic information and background measures for the previous data collected in the afternoon (Study 1) are presented in Table 2.4.1.

No significant differences between treatment groups for any of the background measures or demographic information were observed for children tested in the morning. This suggests that both treatment groups were evenly matched in terms of age, gender, school age group, non-verbal intelligence, sustained attention, reading ability. For both treatment groups, RCPM performance was slightly higher than the norm for children aged eight years (the mean age for both treatment groups; Raven, 1981). Here, the average score was 27 for both treatment groups compared to the RCPM British Percentile norm of 25 reported by Raven (1981) at this age. This suggested that children in Study 2 had higher than average non-verbal intelligence. When considering the CPT percentage commission errors and percentage omission errors, this cohort achieved average percentage errors for both outcome measures compared to the norms (commission error mean = 58 – 68% dependent on gender; omission error mean = 6.5 – 6.7% dependent on gender; Conners et al., 2003). This suggests that these children are performing above their age as these norms were for children aged nine to eleven. Last, both groups performed within one standard deviation above the norm for their age group for all YARC components: reading accuracy, reading rate, reading comprehension, SWRT. This indicated that all children had average to good reading ability and were matched to the children in Study 1 for reading accuracy, reading rate, and reading comprehension.

When comparing background measures and demographic information from the morning and afternoon data sets, a significant difference of time of day was observed for YARC reading rate (p = .034). Here, those tested in the afternoon had a faster reading rate compared to those tested in the morning, regardless of treatment. However, this difference in YARC reading rate did not extend to YARC reading accuracy or YARC reading comprehension which were not significantly affected by time of day.

Further, a significant difference was observed for time of day for CPT omissions (p < .001), regardless of treatment. Here, fewer omission errors were made in the morning compared to the afternoon. A significant difference was also observed between time of day for CPT

commissions ($p < .001$) where a greater percentage of commission errors were apparent in the morning, regardless of treatment, compared to the afternoon.

No significant differences were observed between time of day or treatment group for non-verbal IQ, YARC reading accuracy, YARC reading comprehension, or SWRT.

	Placebo (*n* = 30)			Blueberry (*n* = 31)				
	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Age	8.12	0.83	7.00-10.04	8.22	.77	7.03-10.01	-.467	.624
Gender (male:female)^	16:14	---	---	15:16	---	---	---	---
School Year (3:4)	18:13	---	---	16:14	---	---	---	---
Continuous Performance Task								
Omissions (%)	6.66	4.57	0-19.16	5.62	3.65	.41-15.80	.976	.333
Commissions (%)	67.12	17.10	36.66-90	62.89	19.13	23.33-90	.901	.371
Raven's Coloured Progressive Matrices	27.23	4.31	19-36	27.87	4.70	18-35	-.544	.589
York Assessment of Reading for Comprehension								
Reading Accuracy								
Raw scores	55.25	10.02	34-71	57.03	7.01	41-71	---	---

	Standard scores	107.78	12.20	70-126	108.97	8.95	84-130	-.422	.674

Table 3.4.1: Data for background measures and demographics including raw score means, standard score means, standard deviations, range, *t* statistics, and *p* values for participants tested in the morning.

Table 3.4.1: *continued…*

	M	*SD*	Range	*M*	*SD*	Range	*t*	*p* values
Reading Rate								
Raw scores (ms)	73.86	13.02	41-95	76.28	13.13	47-96	---	---
Standard scores	113.70	13.66	76-130	117.61	11.51	82-130	-1.148	.256
Reading Comprehension								
Raw scores	67.39	7.87	51-81	69.00	6.63	56-83	---	---
Standard scores	117.30	8.32	65-130	119.87	7.61	61-130	-1.219	.228
Single Word Reading Task								
Raw scores	41.55	9.85	14-59	43.55	7.61	21-57	-.883	.381
Standard scores	107.55	15.28	---	109.03	9.91	---	---	---

Notes. York Assessment of Reading for Comprehension standard scores (*M* = 100, *SD* = 15). ^ No significant differences were observed between the number of males and females receiving each treatment (males: $\chi^2 = .15$, $p = .700$, females: $\chi^2 = .86$, $p = .350$)

3.4.2 Cognitive measures

3.4.2.1 AVLT

The general pattern of word list recall performance for both morning and afternoon data sets was similar. Word recall increased between recalls A1-A5 at each test session. After the distractor list B was presented, word recall fell. Further recalls A6 and A7 (short and long delay) demonstrated a decrease in word recall compared to recall A5 thought to be caused by retroactive interference of the learning of list B. The overall pattern of AVLT word recall performance for children tested in the morning are presented below whilst the overall pattern of word recall performance for children tested in the afternoon are presented in Section 2.4.2.1. Means and standard deviations for all AVLT dependent variables are presented in Table 3.4.2.1A.

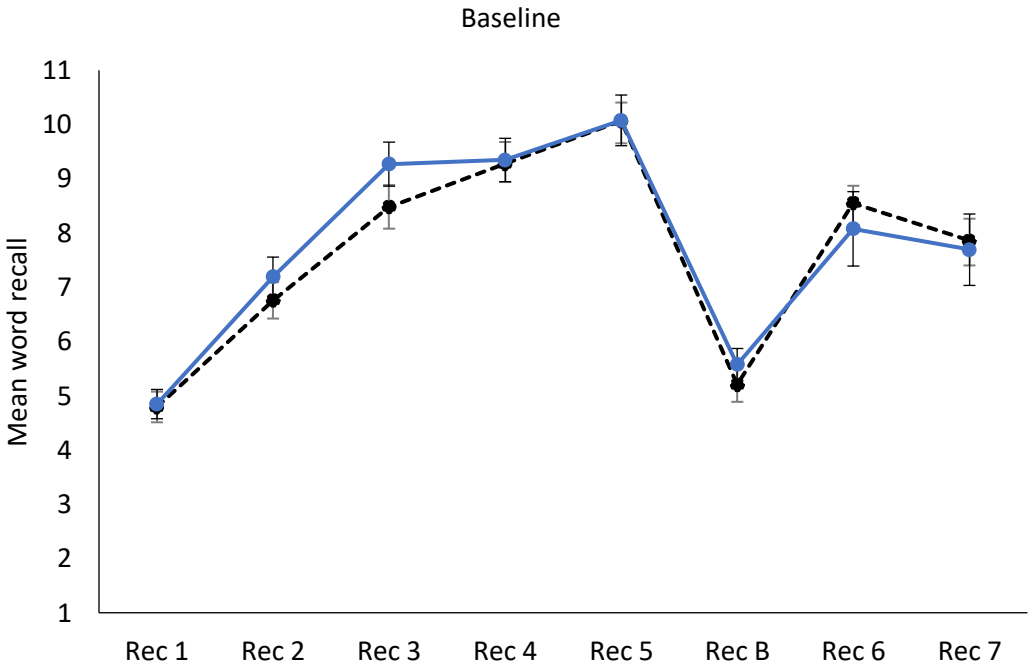
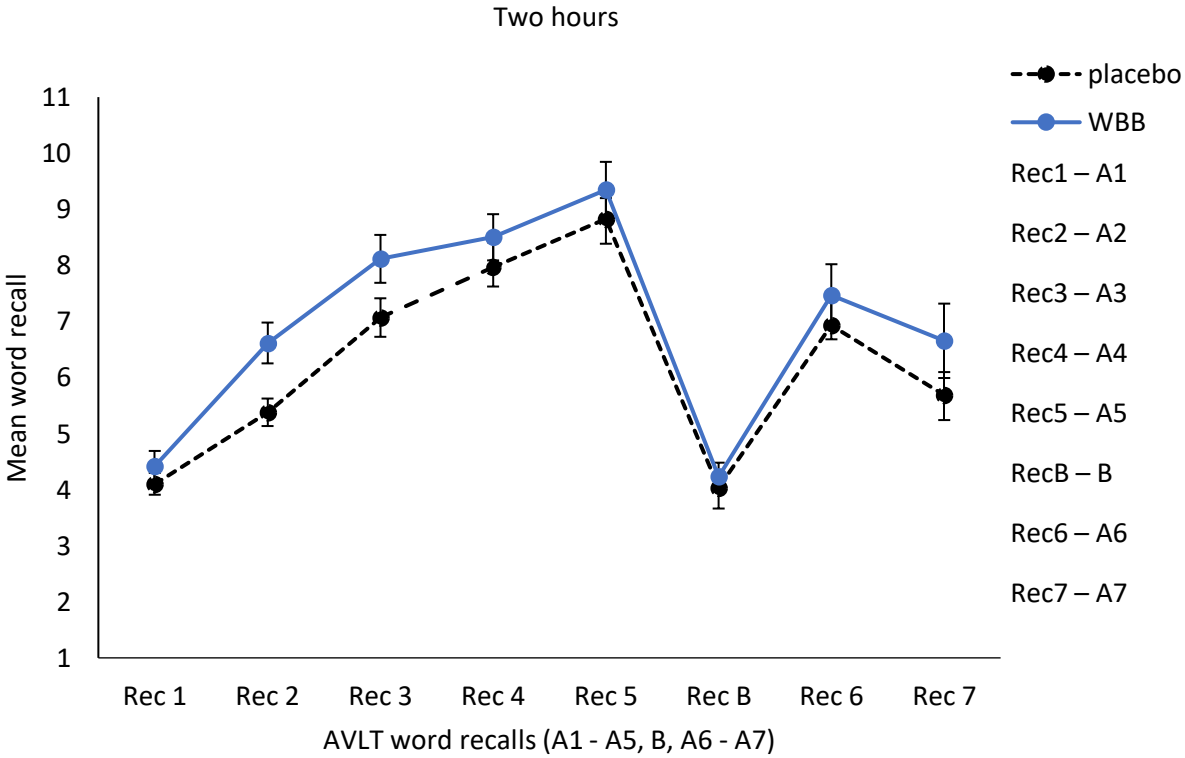


Figure 3.4.2.1A: Mean word recall at each recall point (A1-A5, B, A6-A7) for WBB and



placebo at baseline and two hours post-consumption when children were tested in the morning. Adjusted model means and standard error presented.

Table 3.4.2.1A: Morning and afternoon means and standard deviations for all AVLT dependent variables, including *p* values for differences between word recall performance at baseline and two hours post-intervention for each treatment group.

	Baseline										**Two hours**									
	Morning (*N* = 61)					**Afternoon (*N* = 54)**					**Morning (*N* = 61)**					**Afternoon (*N* = 54)**				
	Placebo (*n* = 30)		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**			**Placebo (*n* = 30)**		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Word Span	4.79	1.52	4.85	1.38	.893	4.38	1.47	4.13	1.23	.419	4.10	1.05	4.42	1.36	.331	4.13	1.23	4.04	1.19	.796
Words Learnt	5.28	2.27	5.23	2.27	.942	5.08	1.99	3.79	1.41	.970	4.72	2.09	4.92	2.62	.756	3.79	1.41	4.11	2.04	.525
Final Acquisition	10.07	2.23	10.08	2.38	.990	9.46	2.64	7.92	1.93	.567	8.83	2	9.35	2.55	.402	7.92	1.93	8.15	2.25	.697
Proactive Interference	-.41	1.82	-.73	1.69	.508	-.17	1.55	.42	1.61	.106	.07	1.58	.19	1.70	.781	.42	1.61	.37	1.21	.908
Retroactive Interference	1.52	1.81	2	2.02	.353	2.5	2.92	1.50	1.98	.110	1.89	1.88	1.89	1.84	.981	1.50	1.98	1.67	1.66	.745

Table 3.4.2.1A: *continued…*

	M	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*
Short Delayed Recall	8.55	2.16	8.08	3.49	.543	6.92	3.01	7.41	2.76	.546	6.93	2.42	7.46	2.85	.458	6.08	2.24	6.48	2.15	.521
Long Delayed Recall	7.86	2.47	7.69	3.36	.831	5.25	2.69	5.81	2.43	.435	5.69	2.19	6.65	3.38	.210	7.04	2.29	6.74	2.10	.627
Total Delayed Recall	16.41	4.43	15.77	6.72	.673	13.96	4.84	14.15	4.68	.887	12.62	4.24	14.12	6.05	.289	11.33	4.26	12.29	4.22	.422
Total Recall	39.38	7.41	40.73	7.92	.516	37.17	9.83	34.85	8.59	.374	33.34	6.89	37	8.09	.076	30.75	7.76	32.11	7.31	.522
Word Recognition	10.83	2.56	10.58	3.79	.773	10.63	3.45	10.33	3.17	.755	9.24	2.69	9.04	3.28	.802	9.21	2.79	9.44	2.03	.729

When assessed independently, the fixed effect of Treatment was a significant predictor of total word recall; F(1,104.62) = .15, MSE = 1.68, $p < .001$, $n^2 < .001$, with pairwise comparisons revealing greater word recall two hours after WBB compared to placebo (p = .009), regardless of time of day.

The fixed effect of Time of Day was a significant predictor of words learnt (A5); F(1,105.71) = 2.36, MSE = .65, p = .032, n^2 = .02, with pairwise comparisons revealing greater word recall in the morning compared to the afternoon (p = .027), regardless of treatment. Time of Day was also a significant predictor of final acquisition; F(1,93.89) = 10.38, $p < .005$, n^2 = .09, with pairwise comparisons revealing a near significant trend for greater word recall in the afternoon compared to the morning (p = .079), regardless of treatment. Further, Time of Day significantly predicted long delayed word recall (A7); F(1,122.92) = 17.73, MSE = .58, $p < .001$, n^2 = .13, with pairwise comparisons revealing significantly greater word recall in the afternoon compared to the morning ($p < .001$), regardless of treatment. Last, Time of Day significantly predicted total delayed word recall; F(1,106) = 15.28, MSE = -2.11, $p < .001$, n^2 = .13, however pairwise comparisons revealed no significant difference in word recall between participants tested in the morning and afternoon (p = .552), regardless of treatment.

Time of Day and Treatment did not predict any other AVLT dependent variables. A near significant Time of Day x Treatment interaction was observed for long delayed word recall; F(1,135.14) = 3.36, MSE = -1.07, p = .069, n^2 = .02. However, further analysis by pairwise comparisons revealed no significant differences between treatments or between morning and afternoon performance (Table 3.4.2.1B). No further Treatment x Time of Day interactions were observed.

Table 3.4.2.1B: β coefficients and *p* values for the fixed effects and interactions for all AVLT LMM analyses at two hours.

	β coefficient	*p* values
Word Span		
Treatment	-.01	.470
Time of Day	.17	.927
Treatment*Time of Day	-.31	.484
Words Learnt		
Treatment	-.31	.497
Time of Day	.78	.032
Treatment*Time of Day	.10	.849
Final Acquisition		
Treatment	.50	.892
Time of Day	-.91	.002
Treatment*Time of Day	-.89	.238
Proactive Interference		
Treatment	-.06	.709
Time of Day	-.21	.364
Treatment*Time of Day	-.11	.856
Retroactive Interference		
Treatment	-.21	.800
Time of Day	.19	.375
Treatment*Time of Day	.24	.742

Table 3.4.2.1B: *continued…*

	β coefficient	*p* values
Short Delayed Recall		
Treatment	-.18	.249
Time of Day	.69	.314
Treatment*Time of Day	-.55	.489
Long Delayed Recall		
Treatment	.46	.564
Time of Day	-1.07	<.001
Treatment*Time of Day	-1.39	.069
Total Delayed Recall		
Treatment	.385	.328
Time of Day	-2.11	< .001
Treatment*Time of Day	-2.26	.140
Total Recall (A1-A5)		
Treatment	-2.69	.008
Time of Day	1.36	.237
Treatment*Time of Day	-.20	.923
Word Recognition		
Treatment	-.40	.779
Time of Day	-.53	.552
Treatment*Time of Day	.57	.488

3.4.2.2 MANT

3.4.2.2.1 Accuracy

Consistent with previous research (Whyte et al., 2016; 2017), both morning and afternoon data sets exhibited greater accuracy for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials), regardless of treatment. For both data sets, the fixed effect of Congruency was a significant predictor of accuracy; F(1,452.87) = 24.58, MSE = .01, $p < .001$, $n^2 = .05$, with pairwise comparisons revealing greater accuracy on congruent trials compared to incongruent trials. The fixed effect of Target Time was also a significant predictor of accuracy; F(1,393.98) = 35.37, MSE = -.06, $p < .001$, $n^2 = .08$, with pairwise comparisons revealing greater accuracy on 500 ms trials compared to 120 ms trials. Load also significantly predicted accuracy; F(1,767.07) = 5.63, MSE = .02, $p = .018$, $n^2 = .08$, with pairwise comparisons revealing greater accuracy on medium load trials compared to high load trials. In general, accuracy performance was high with participants gaining between 77-91% of correct responses at two hours. Means and standard deviations are presented in Table 3.4.2.2.1A.

Table 3.4.2.2.1A: Morning and afternoon means and standard deviations for MANT accuracy, including *p* values for differences between word recall performance at baseline and two hours post-intervention for each treatment group.

	Baseline										**Two hours**									
	Morning (*N* = 61)					**Afternoon (*N* = 54)**					**Morning (*N* = 61)**					**Afternoon (*N* = 54)**				
	Placebo (*n* = 30)		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency																				
Congruent	.88	.11	.87	.10	.589	.83	.15	.83	.13	.929	.88	.07	.91	.07	.729	.87	.16	.86	.15	.533
Incongruent	.74	.16	.75	.14	.494	.67	.19	.68	.19	.827	.84	.13	.87	.12	.839	.77	.18	.77	.17	.999
Load																				
High	.81	.13	.80	.12	.610	.74	.15	.74	.16	.878	.86	.09	.89	.10	.945	.81	.16	.78	.15	.780
Medium	.82	.13	.82	.11	.423	.76	.17	.77	.14	.977	.87	.09	.89	.09	.985	.839	.17	.83	.15	.738
Target Time																				
120 ms	.81	.14	.80	.12	.818	.72	.18	.72	.17	.893	.84	.12	.87	.11	.790	.79	.19	.79	.17	.797
500 ms	.81	.13	.82	.12	.241	.78	.15	.78	.14	.972	.89	.09	.91	.08	.828	.86	.15	.84	.14	.722

Although there was no Time of Day x Treatment interaction for either Congruency, Load or Target Time, the LMM MANT accuracy analysis revealed that the fixed effect of Time of Day, when assessed independently, was a significant predictor of executive function accuracy; F(1,101.39) = 4.11, MSE = .05, p = .045, n^2 = .04. Pairwise comparisons revealed that participants tested in the morning had greater accuracy than those tested in the afternoon, regardless of treatment.

The fixed effect of Treatment did not predict MANT accuracy and no Treatment x Time of Day interaction was observed (Table 3.4.2.2.1B).

Table 3.4.2.2.1B: β coefficients and *p* values for the fixed effects for the MANT accuracy LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
Treatment	< .005	.764
Time of Day	.05	.045
Congruency	.03	< .001
Load	-.03	.018
Target Time	-.04	< .001
Treatment*Time of Day	-.03	.253
Time of Day*Treatment*Congruency	-.04	.902
Time of Day*Treatment*Load	.01	.808
Time of Day*Treatment*Target Time	-.01	.839

3.4.2.2.2 Reaction time for correct responses only (ms)

In line with Whyte et al. (2017), both morning and afternoon data sets revealed faster reaction times for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials) at two hours, regardless of treatment. For both data sets, the fixed effect of Congruency was a significant predictor of reaction time; F(1,824.46) = 153.07, MSE = -47.91, p < .001, n^2 = .16,

with pairwise comparisons revealing faster reaction times for congruent trials compared to incongruent trials ($p < .001$). Similarly, the fixed effect of Load was a significant predictor of reaction time; F(1,767.78) = 16.09, MSE = 8.74, $p < .001$, $n^2 = .02$, with pairwise comparisons revealing faster reaction times for medium load trials compared to high load trials ($p < .001$), regardless of treatment and session. Target Time did not significantly predict reaction time for either data set. Means and standard deviations are presented in Table 3.4.2.2.2A.

	Baseline										Two hours									
	Morning (*N* = 61)					Afternoon (*N* = 54)					Morning (*N* = 61)					Afternoon (*N* = 54)				
	Placebo (*n* = 30)		WBB (*n* = 31)			Placebo (*n* = 25)		WBB (*n* = 29)			Placebo (*n* = 30)		WBB (*n* = 31)			Placebo (*n* = 25)		WBB (*n* = 29)		
	M	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*	*M*	*SD*	*M*	*SD*	*p value*
Congruency																				
Congruent	580.05	99.29	613.43	90.76	.194	549.79	89.75	529.21	113.63	.469	585.59	112.76	596.21	83.46	.395	544.84	65.88	513.08	116.79	.234
Incongruent	655.83	114.87	676.65	97.77	.465	621.36	122.26	585.81	155.91	.361	617.51	109.65	642.79	92.35	.229	618.15	83.04	579.07	141.42	.231
Load																				
High	631.06	113.23	658.48	95.19	.332	594.09	106.50	565.48	138.11	.404	608.74	112.35	627.13	92.06	.305	588.59	78.584	554.82	133.27	.272
Medium	604.82	99.58	612.25	87.73	.291	577.06	101.79	548.69	129.39	.380	594.37	107.07	611.86	82.71	.291	554.82	72.205	537.35	123.21	.193

Target Time																			
120 ms	603.43	92.81	635.13	87.16	.192	550.24	118.09	530.94	140.81	.591	596.82	109.49	621.12	84.65	.166	573.69	77.019	529.55	137.25	.160
500 ms	632.45	126.17	654.95	104.68	.472	620.91	100.92	583.04	129.70	.363	606.29	118.55	617.87	95.77	.501	589.30	86.901	562.46	121.92	

Table 3.4.2.2.2A: Morning and afternoon means and standard deviations for MANT reaction time (ms; correct responses only) at baseline and two hours post-consumption.

The analysis revealed a significant Treatment x Time of Day x Target Time interaction; F(2,761.68) = 4.37, MSE = 8.83, p = .013, n^2 = .01. Pairwise comparisons revealed a significant difference of Time of Day for 120 ms trials for those consuming WBB only (p = .006). Specifically, participants tested in the afternoon had significantly faster reaction times two hours after WBB supplementation compared to those tested in the morning and compared to placebo when given in the afternoon (Figure 3.4.2.2.2).

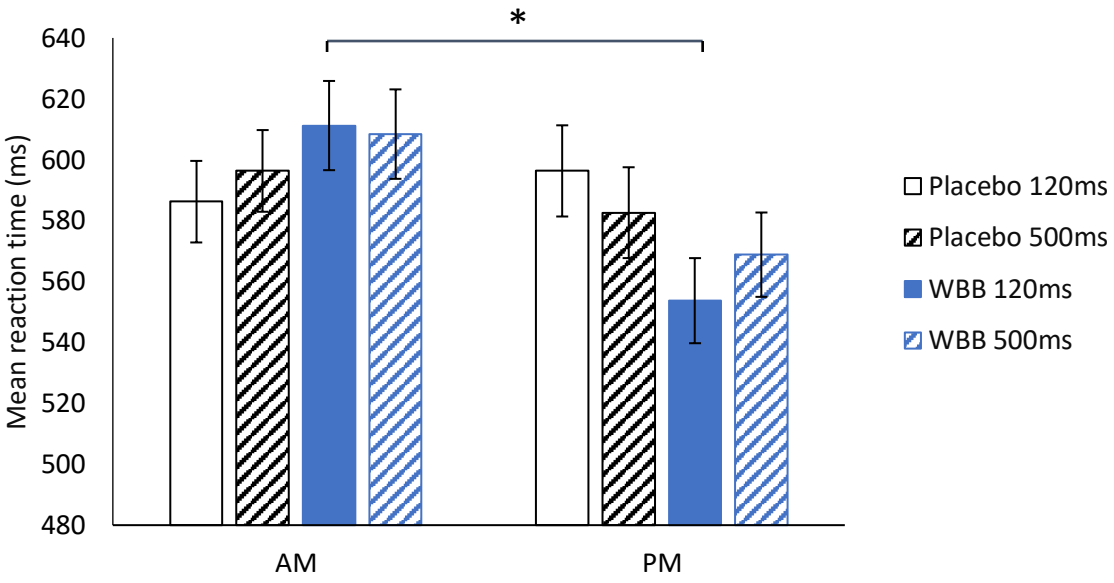


Figure 3.4.2.2.2: Significant Treatment x Time of Day x Target Time interaction for reaction time (ms*; p* =.013). Pairwise comparisons revealed a significant difference of Time of Day for 120 ms trials (p = .006) for the WBB group only. Faster reaction times were observed in the afternoon two hours following WBB consumption compared to the morning and to placebo when given in the afternoon. Adjusted model means and standard error presented. LMM with baseline as a covariate.

The fixed effects of Treatment was not a significant predictor of reaction time when assessed independently. However, a near significant trend for Time of Day was observed; F(1,97.34) = 3.60, MSE = 21.18, p = .061, n^2 = .04, with pairwise comparisons revealing no significant difference between reaction times, regardless of treatment and session. There were no other significant interactions between the remaining MANT parameters, Time of Day, or Treatment (Table 3.4.2.2.2B).

Table 3.4.2.2.2B: β coefficients and *p* values for the fixed effects for MANT reaction time (ms; correct responses) LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
Treatment	12.15	.797
Time of Day	38.26	.061
Congruency	-47.91	< .001
Load	8.74	< .001
Target Time	-17.54	.294
Treatment*Time of Day	-32.82	.145
Time of Day*Treatment*Congruency	-20.33	.226
Time of Day*Treatment*Load	6.65	.661
Time of Day*Treatment*Target Time	-18.79	.013

3.4.2.3 PANAS-C

The overall pattern from the combined data set revealed that participants had greater self-reported feelings of positive affect and lower self-reported feelings of negative affect. Means and standard deviations are presented in Table 3.4.2.3A.

Table 3.4.2.3A: Morning and afternoon means and standard deviations for positive affect and negative affect at baseline and two hours post-consumption.

	Baseline										**Two hours**									
	Morning (*N* = 61)					**Afternoon (*N* = 54)**					**Morning (*N* = 61)**					**Afternoon (*N* = 54)**				
	Placebo (*n* = 30)		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**			**Placebo (*n* = 30)**		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	**p value**
Positive affect	49.84	11.37	52.37	12.17	.405	49.21	10.04	49	11.96	.116	51.48	10.03	53.80	12.51	.442	48.16	11.654	52.83	9.831	.947
Negative affect	16.45	1.79	17.30	5.83	.427	19.25	3.86	20.79	7.43	.685	16.61	2.43	17.17	6.01	.637	20.76	7.230	19.9	7.61	.366

The fixed effect of Time of Day significantly predicted positive affect; F(1,113) = 6.64, MSE = 1.16, p = .012, n^2 = .06, with pairwise comparisons revealing significantly greater self-reported positive affect in the morning compared to the afternoon (p = .007), regardless of treatment. Time of Day did not predict negative affect.

A near significant Treatment x Time of Day interaction was observed for positive affect; F(1,113) = 2.81, MSE = 2.27, p = .096, n^2 = .02, with pairwise comparisons revealing significantly greater positive affect following acute WBB intake in the morning compared to the afternoon (p = .003).

The fixed effect of Treatment did not predict positive or negative affect. No Treatment x Time of Day interaction was observed for negative affect (Table 3.4.2.3B).

Table 3.4.2.3B: β coefficients and *p* values for the fixed effects for positive affect and negative affect LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
Positive Affect		
Treatment	3.59	.143
Time of Day	4.79	.012
Treatment*Time of Day	-3.80	.096
Negative Affect		
Treatment	-1.37	.358
Time of Day	-1.97	.103
Treatment*Time of Day	1.32	.391

3.4.3.4 TOWRE-2

In general, both morning and afternoon data sets observed greater TOWRE-2 sight word efficiency (SWE) performance than phonemic decoding efficiency (PDE) at two hours whereby participants read aloud more words correctly than non-words. This is consistent

with the expected pattern of performance where children read aloud words at a greater rate than to non-words, especially when comparing known versus novel orthography. This explains why fewer non-words were read aloud in the PDE task as more time would be needed for decoding the novel orthography. Means and standard deviations are present in Table 3.4.3.4A.

Table 3.4.3.4A: Morning and afternoon means and standard deviations for SWE and PDE at baseline and two hours post-consumption.

	Baseline										**Two hours**									
	Morning (*N* = 61)					**Afternoon (*N* = 54)**					**Morning (*N* = 61)**					**Afternoon (*N* = 54)**				
	Placebo (*n* = 30)		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**			**Placebo (*n*= 30)**		**WBB (*n* = 31)**			**Placebo (*n* = 25)**		**WBB (*n* = 29)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
SWE	64.52	13.26	66.43	12.59	.565	60.67	13.06	64.74	12.18	.255	64.42	13.46	67.43	12.58	.679	61.42	13.63	65.70	12.06	.239
PDE	31.48	12.22	32.70	10.51	.370	30.46	12.37	33.93	9.52	.265	31.26	11.93	33.70	10.29	.396	31.58	12.99	34.48	9.99	.373

The fixed effects of Treatment and Time of Day did not significantly predict either SWE or PDE, and no Time of Day x Treatment interactions were observed (Table 3.4.3.4B).

Table 3.4.3.4B: β coefficients and *p* values for fixed effects for the SWE and PDE LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
SWE		
Treatment	-.45	.351
Time of Day	.13	.779
Treatment*Time of Day	-.76	.665
PDE		
Treatment	.29	.556
Time of Day	.35	.593
Treatment*Time of Day	-1.62	.349

3.5 Discussion

The present study aimed to examine a potential interaction between time of day and acute WBB supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds. The study examined the effects of acute WBB supplementation on performance by combining two data sets where performance was assessed in the morning (Study 2) and the afternoon (Study 1). This was to allow for the direct comparison of the effects of acute WBB supplementation on performance at different times of day through the analysis of Treatment x Time of Day interactions. The study observed significantly faster executive function reaction times for the more cognitively demanding trials two hours after WBB supplementation in the afternoon, compared to the morning and to placebo. Overall, acute WBB intake significantly improved total word recall (A1 – A5) compared to placebo, regardless of time of day. Time of day had a significant effect on performance for: final acquisition (A5), executive function accuracy, and positive affect, with greater performance observed in the morning compared to the afternoon, regardless of treatment. In contrast,

time of day had a significant effect on delayed word recall (A7) with greater performance observed after 25 minutes in the afternoon compared to the morning, regardless of treatment.

The significant Treatment x Time of Day x Target Time interaction for executive function reaction time (Section 3.4.2.2.2) suggests that acute WBB supplementation may be able to aid children's processing speed for the more cognitively demanding trials in the afternoon. This time of day is when cognitive performance is seen to naturally decrease (Cerasuolo et al., 2016) as a result of a decrease in alertness and an increase in sleep pressure. As no effect of treatment was observed for the less cognitively demanding trials, this suggests that acute WBB consumption may help direct attention to tasks that place greater cognitive demand on resources. In particular, these improvements are further observed during times of cognitive fatigue, such as in the afternoon where performance is naturally declining; no effect of acute WBB supplementation was observed in the morning for executive function. This finding supports Whyte et al. (2017) where a significant improvement to executive function reaction time was observed three hours (afternoon test session) after acute WBB supplementation for the more cognitively demanding high load trials. However, these significant interactions were apparent for different MANT parameters between the two studies (Study 2: target time; Whyte et al., 2017: load). As Study 2 is one of only three studies to observe significant acute WBB effects on improving executive function reaction times for more cognitively demanding trials in children (Study 1; Whyte et al., 2017), future studies are needed to establish which MANT parameters are consistently benefitting from acute WBB treatments for the more cognitively demanding trials in general.

Time of day independently predicted performance on a number of outcome measures: final acquisition (A5), long delayed word recall (A7), executive function accuracy, positive affect. In general, those tested in the morning had greater final acquisition, executive function accuracy, and positive affect, than those tested in the afternoon, regardless of treatment. The opposite was observed for total delayed word recall only where a greater number of words were recalled in the afternoon compared to morning. The findings for improved performance in the morning are in line with previous research (Janvier & Testu, 2007; Cerasuolo et al., 2016) examining fluctuations in cognitive performance over a 24-hour period where greater performance was observed in the morning, followed by a decrease in performance in the afternoon. Although final acquisition showed superior recall in the

morning, word recall after a long delay (25 minutes) showed superior recall in the afternoon; a finding in contrast to the general pattern of daily cognitive fluctuations. However, it is important to note that time of day only predicted performance for two out of the ten AVLT measures, thus potential spurious findings could be the result of type I error. For executive function, accuracy performance in general was greater in the morning compared to the afternoon. This contrasts to the effect of time of day on executive function reaction times which were faster in the afternoon (compared to the morning) specifically for the more cognitively demanding trials. Time of day did not independently predict reaction times. Despite these isolated findings, it is difficult to draw a clear conclusion about the consistent impact of time of day in this study. Indeed, the time of day effects observed in Study 2 may be a consequence of testing two different cohorts of children across the two studies, where the differences in background and demographic measures (reading and selective attention) observed may have influenced the results. This highlights the need for future research to replicate the present study in a single sample of children (thus eliminating differences in cohort background and demographic measures) to examine time of day effects on these cognitive measures to create a consistent pattern of results.

Although no two-way interactions were observed for the AVLT measures, a near significant Time of Day x Treatment interaction was observed for long delayed word recall; $F(1,135.14) = 3.36$, MSE = -1.07, $p = .069$, $n^2 = .02$, however pairwise comparisons failed to reveal a significant difference between treatment groups or time of day. As this was the only interaction to reach near significance, this finding may be the result of type 1 error, resulting in a false positive result. Further, this interaction may have reached significance if a large sample size was recruited. Although the study was adequately powered and recruited an appropriate sample size in line with the *a priori* power calculation, it is possible that a larger sample size could have yielded greater power and thus resulted in a significant interaction.

An independent assessment of Treatment showed that this fixed effect significantly predicted total word recall (A1-A5) with greater word recall observed two hours after WBB consumption compared to placebo, regardless of time of day. This is in line with previous research (Study 1; Whyte & Williams, 2015; Whyte et al., 2016) where acute WBB intake improved word list recall one to two hours after treatment. From the present study, a consistent effect of acute WBB consumption on word list recall has emerged from the current child literature. This suggests that acute WBB supplementation is able to aid

encoding and retrieval processes over repeated stimuli presentation involved in word learning assessed by word list recall, as a potential result of increasing alertness levels and sustained attention to the stimuli.

Despite the previous study (Study 1) observing significant improvements to positive affect two hours after acute WBB consumption, WBB intake did not significantly predict mood or interact with time of day in the present study. However, Time of Day independently predicted positive affect with greater self-reported positive affect observed in the morning compared to the afternoon, regardless of treatment. This fluctuation in mood is in line with fluctuations in cognitive functioning, further supporting shared mechanisms of action between cognition and mood. However, this contrasts to the literature examining time of day effects on mood. Diaz-Morales et al. (2016) assessed the mood of adolescents (aged 12-16 years) in the early morning (0810 – 0830 hours), late morning (1020 – 1140 hours), and early afternoon (1350 – 1410 hours). Positive affect was reported to be at its lowest in the early morning, increasing throughout the day with greatest positive affect in the early afternoon. This is supported by Murray et al. (2002) who demonstrated positive mood fluctuations over a 24-hour period. Positive affect was shown to peak in the early afternoon, followed by a plateau and further decline in the late evening; this fluctuation was not observed for negative affect.

It is important to note that limited research has examined the effect of time of day on school-aged children's mood. Instead the majority of research (Diaz-Morales et al., 2016; Murray et al., 2002) has focused on time of day fluctuations on the mood of adolescents and adults. This helps to explain why there is a discrepancy between the mood findings observed here, and those reported in the previous literature. One explanation for changes in children's mood throughout the day could be due to morningness and eveningness chronotypes (Diaz-Morales et al., 2016). Those with a morningness chronotype tend to wake earlier and be more alert and attentive in the morning whilst those with an eveningness chronotype tend to perform better in the evening compared to the morning, where performance and mood are usually worse (Vink et al., 2001). Morningness and eveningness chronotypes are thought to fluctuate and undergo developmental changes throughout the human lifespan (Adan et al., 2012). Morningness is thought to be the norm in early development with a shift towards eveningness as children approach adolescence, followed by a final shift back to morningness in adulthood. This fluctuation in morningness and

eveningness chronotypes whereby children are more alert, attentive, and experience greater positive affect in the late morning to early afternoon may explain why the present mood data differs to that found previously in adolescents and adults (Diaz-Morales et al., 2016; Murray et al., 2002).

Despite a connection between the aspects of cognition known to benefit from acute WBB consumption and reading behaviour, no effects of treatment were observed for either SWE or PDE. Further, no significant differences for SWE and PDE were observed between morning and afternoon testing indicating that reading performance remained consistent throughout the school day. In parallel to the research in Study 1, the present findings suggest that an acute WBB treatment may not be enough to elicit improvements to reading behaviour, thus prompting future research to examine the effects of chronic WBB treatments on reading behaviour. This is supported by the previous PUFAs literature which observes significant improvements to reading behaviour following chronic supplementation only (Johnson et al., 2017; Dalton et al., 2009). In addition, these findings support the notion that the TOWRE-2 may lack sensitivity to identify improvements following a dietary intervention. Hence, both studies (Study 1 & Study 2) collectively suggest the use of a more sensitive measure assessing the effects of acute WBB treatment on a specific aspect of reading (Section 2.5).

The baseline differences observed for the CPT findings between those tested in the morning and those tested in the afternoon suggest that participants were able to pay greater attention to the task stimuli in the morning, compared to the afternoon, regardless of treatment. In the morning, participants continued to identify coloured circles correctly, although a greater number of commission errors suggests that participants had trouble differentiating between target circles and distractor circles, responding to both stimuli as targets. However, participants made greater omission errors when tested in the afternoon compared to the morning, regardless of treatment. This suggests a lack of attention to the task at this time of day as they responded to fewer circles in general, whether target or foil. These findings suggest participants had different levels of attention at the different times of day, resulting in a detriment to inhibition in the morning and a detriment to overall attention in the afternoon. However, it is again important to note that two different cohorts of children were recruited in Study 1 and Study 2, therefore differences in background and demographic measures may be expected and may explain the differences observed. Overall, these findings support children's cognitive fluctuations observed throughout the day where

levels of alertness are greater in the morning compared to the afternoon (Cerasuolo et al., 2016). Further, these baseline differences in attention may have contributed to the time of day effects observed for word list recall, executive function accuracy, and positive affect which demonstrated greater performance in the morning compared to the afternoon. These baseline differences in attention provide support for the idea that acute WBB supplementation improves cognitive performance during times of cognitive fatigue. Indeed, executive function reaction times were significantly faster in the afternoon two hours after WBB intake (for the more cognitively demanding trials), whilst performance following the placebo was reduced; in line with the reduction in levels of attention observed by the CPT. Therefore, acute WBB supplementation was able to overcome cognitive fatigue for these more cognitively trials.

The present results from this time of day comparison study provide clarification on the subtle differences observed between the previous study (Study 1) and Whyte et al. (2016). The present study observed a significant improvement to executive function reaction time two hours after WBB supplementation for the more cognitively demanding trials; this is in contrast to Whyte et al. (2016) who observed a significant improvement to executive function accuracy three hours after treatment consumption when tested in the afternoon. Instead, the improvement to executive function reaction time observed in the present study is similar to that found by Whyte et al. (2017) where acute WBB supplementation induced faster reaction times three hours after treatment consumption again for the more cognitively demanding trials when assessed in the afternoon. Further, the present study was able to extend the word list recall findings from Study 1, specifically for total word recall, with greatest improvements observed two hours after WBB supplementation. This finding can also support the significant improvement to immediate word recall (A5) two hours after WBB intake observed by Whyte et al. (2016). This highlights a consistent effect of acute WBB treatment on word list recall.

To conclude, time of day significantly interacted with acute WBB supplementation for executive function reaction time. Here, faster reaction times for the more cognitively demanding 120 ms fast trials were observed in the afternoon two hours after WBB consumption compared to the less cognitively demanding 500 ms slow trials, to morning performance, and to placebo. Although no other treatment by time of day interactions were observed, the study highlighted a significant effect of treatment for total word list recall (A1

– A5) with a greater number of words recalled two hours after acute WBB intake compared to placebo, regardless of time of day. Further, a significant effect of time of day was observed for final acquisition with a greater number of words recalled in the morning compared to the afternoon, whilst long delayed word recall (A7) showed a greater number of words recalled in the afternoon compared to the morning. Last, time of day significantly predicted mood with greater positive affect observed in the morning compared to the afternoon, regardless of treatment. However, these time of day effects should be taken with caution as two different cohorts of children were recruited for Study 1 and Study 2. Therefore, the time of day effects observed here may be due to differences between participants recruited for the morning and afternoon studies.

This study adds to the previous literature in children conducted thus far for word list recall (Study 1; Whyte et al., 2016) and for executive function reaction times (Study 1; Whyte et al., 2017). In this instance, there was no effect of treatment or time of day on reading behaviour. The findings here are consistent with the previous acute study (Study 1) and further supports the need for exploration of the effects of chronic WBB supplementation on reading behaviour. Indeed, the previous PUFAs literature (Johnson et al., 2017; Dalton et al., 2009) demonstrated improvements to reading behaviour following chronic supplementation only, suggesting that the complex set of cognitive skills involved in reading behaviour may be sensitive to the accumulative effects of dietary interventions consumed over a prolonged period rather than a single, one-off dose of treatment.

Following on from the present study, Study 3 will examine the effects of chronic WBB supplementation on the cognition, reading behaviour, and mood, of seven to ten-year olds. This study will examine performance over a four-week supplementation period, incorporating an acute test day with two further chronic test days at weeks two and four.

Chapter 4

Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds.

4.1 Introduction

Previous research highlighted the acute benefits of WBB supplementation on children's word list recall, executive function, and mood, up to six hours after treatment (Whyte & Williams, 2015; Whyte et al., 2016; 2017; Studies 1 & 2). Furthermore, the findings from Study 2 demonstrated an interaction between time of day and acute WBB intake on cognitive performance. Here, executive function reaction time (ms; correct responses only) for the more cognitive demanding fast trials was significantly faster two hours after WBB consumption in the afternoon, compared to the morning and to the placebo group in the afternoon. This finding, along with that observed by Whyte et al. (2016; 2017), suggests that acute WBB supplementation aids executive function performance during periods of cognitive fatigue, such as when tested in the afternoon, and when a greater level of cognitive demand is required. Alongside this interaction, acute WBB supplementation significantly improved total word list recall compared to the placebo, regardless of time of day. Further, self-reported positive affect was significantly greater in the morning compared to the afternoon, regardless of treatment. However, despite clear improvements to the cognitive domains that underpin reading, no improvements to reading behaviour were observed following acute WBB supplementation (Studies 1 & 2).

4.1.1 Chronic WBB supplementation and the benefits to cognition

One potential explanation for the lack of acute WBB effects on reading behaviour seen thus far is that an acute, single dose of WBB may not be enough to elicit positive benefits to reading behaviour. To date, acute WBB treatments have significantly improved performance on the cognitive domains thought to underpin reading, such as memory, attention, and executive function. These findings have been observed when the domains of interest were assessed individually, but not when assessed simultaneously such as for reading behaviour; these cognitive domains must work together simultaneously to accomplish fluent and efficient reading (Sesma et al., 2009). To date, the previous research demonstrates a null effect of acute WBB supplementation on reading behaviour, suggesting that the

simultaneous workings of these multiple cognitive domains of interest when assessing reading behaviour may not be sensitive to acute WBB intake.

As noted previously (Section 3.5), it would be prudent to examine the effect of chronic WBB supplementation on children's cognition, reading behaviour, and mood, to assess the accumulative effects of berry-flavonoids on performance. To date, the effects of chronic berry-flavonoid interventions on children's cognition has not been examined. However, the previous adult literature demonstrates an array of positive benefits to the cognition of both young and older healthy adults following chronic berry-flavonoid supplementation. Bowtell et al. (2017) demonstrated a significant improvement to working memory performance on a 2-back task when young adults were supplemented with a daily 30 ml WBB treatment (387 mg/d anthocyanins) for 16 weeks. Alongside this cognitive benefit, physiological improvements to cerebral blood flow in task-related brain regions were observed whilst undergoing fMRI, thus supporting the effect of chronic WBB supplementation on working memory. Similarly, Nilsson et al. (2017) also observed significant improvements to verbal working memory when healthy 50 to 70-year olds received a daily 350 g mixed berry treatment (approximately 414 mg/d anthocyanins) over a five-week period. Further, Miller et al. (2019) observed significant improvements to older adult's (aged 60-70 years) executive function accuracy on a Task Switching Task after 90 days of a 24 g/d BB treatment (19.2 mg/g anthocyanins). These studies in healthy adults highlight the potential for chronic WBB interventions to improve children's performance on similar aspects of cognition, as well as indicating the potential for such findings to extend to reading behaviour.

4.1.2 Chronic dietary interventions and reading behaviour

Research examining the effects of chronic dietary interventions on children's reading behaviour has focused on assessing daily PUFAs consumption on performance. Indeed, the majority of these studies observed significant reading improvements following chronic PUFAs supplementation. Research demonstrated that children with behavioural and learning difficulties such as developmental coordination disorder (DCD) significantly increased their reading age by nine months following three-month daily fish oil capsules containing omega-3 and omega-6 fatty acids. This increase was in comparison to those receiving the placebo where reading age only increased by three months. The increase was still apparent at a six-month follow-up (Richardson & Montgomery, 2005). When examining the impact of PUFAs

supplementation in typical but underperforming children, Richardson et al. (2012) observed that those who performed 10-20% below aged-matched peers at baseline had a significantly greater improvement in their reading following 16-week daily DHA supplementation. This cohort of the treatment group showed an additional .8-1.9 month mean increase in reading age; this increase was observed in addition to the expected increase in reading performance over this 16-week period. This was in comparison to the placebo group who showed no increase in reading performance attributable to chronic PUFAs supplementation.

Thus far, the research suggests that chronic dietary interventions can have an effect on reading behaviour for those with behavioural and learning difficulties, and those who may be underperforming initially. However, such findings cannot generalise to typical populations where performance is already within the average range for their respective age-group. A recent study by Johnson et al. (2017) examined the effect of daily omega-3 and omega-6 fatty acids over a three-month period on the reading ability of typically developing nine to ten-year olds; all were performing within the normed range. At three-months, significant improvements to phonemic decoding time and visual analysis time (The Logos Test: Hoien, 2007) were seen compared to those consuming the placebo. Those consuming the PUFAs treatment significantly reduced the time spent decoding non-words as well as reducing the time spent distinguishing between correct and incorrect letters. These findings highlight the role of chronic dietary interventions on improving reading behaviours. Therefore, as reading behaviour is sensitive to chronic PUFAs interventions, this further highlights the potential for other chronic dietary interventions, such chronic WBB supplementation, to affect reading behaviour; especially as the effects of chronic WBB supplementation on cognition in adults are already established.

4.1.3 The present study

The present study aimed to examine the chronic effects of a 30 g freeze-dried WBB powder treatment consumed daily over a four-week period on the cognition, reading behaviour, and mood, of seven to ten-year olds. An acute-on-chronic, between groups, randomised, single blind, placebo-controlled study design was implemented with participants randomly assigned to receive either the 30 g freeze-dried WBB powder treatment or a sugar and vitamin-C matched placebo. The acute procedure used previously (Studies 1 & 2) was employed for the acute test day assessing performance at baseline and

two hours after initial treatment consumption, with additional chronic test sessions at two weeks and four weeks. Participants completed the same tasks of word list recall and recognition, executive function, reading behaviour, and mood. Matched versions of these tasks were completed for each test session.

To align with the previous acute research (Studies 1 & 2; Whyte & Williams, 2015; Whyte et al., 2016; 2017), it was hypothesised that acute WBB supplementation would improve total word recall and delayed word recall, executive function, and positive mood, at two hours post-treatment, compared to placebo. Next, consistent with the previous chronic WBB interventions studies (Bowtell et al., 2017; Miller et al., 2019; Nilsson et al., 2017), it was hypothesised that chronic WBB supplementation would further improve total word recall and delayed word recall, executive function, and positive mood, at two weeks and four weeks, compared to placebo. It was further hypothesised that both acute and chronic WBB consumption would aid performance on the more cognitively demanding executive function trials in comparison to the less cognitively demanding trials, and in comparison to the placebo group; this is in line with the pattern of executive function performance observed previously (Studies 1 & 2, Whyte et al., 2016; 2017). Last, regarding the current lack of acute WBB effects observed for reading behaviour, it was hypothesised that acute WBB supplementation would not affect reading behaviour, but that chronic WBB supplementation might positively benefit both sight word efficiency and phonemic decoding efficiency. This hypothesis was based on cognitive findings from the chronic WBB literature where the cognitive domains known to underpin reading improve following chronic WBB supplementation (Studies 1 & 2; Whyte & Williams, 2015; Whyte et al., 2016; 2017). Further, this hypothesis was also based on findings from the PUFAs literature where chronic PUFAs supplementation significantly improved reading behaviour in typically developing children performing within the normed range for their age (Johnson et al., 2017).

4.2 Methods

The present study (for ethical approval, see Appendix A.1) employed a similar methodology to that used previously in Studies 1 and 2. Following a baseline test session, acute testing took place two hours after an initial treatment. Two novel chronic test sessions were then completed at two weeks and four weeks following daily WBB consumption over this four-week period.

4.2.1 Participants

An *a priori* power analysis (using G Power 3.1) based on the significant acute AVLT total word recall finding from Study 1 was used to calculate the required sample size. A two-tailed test with a partial alpha of $p = .05$ revealed a total of 50 children would be required to achieve sufficient power of 0.8 ($n = 21$ per treatment group) when employing a between-subjects design. To date, no studies have examined the effect of chronic WBB supplementation on children's cognition. Therefore, the *a prior* power analysis detailed above was also used to calculate the required sample size for the chronic arm of this study.

Due to difficulties with recruitment, a total of 30 healthy children (12 males: 18 females) aged seven to ten years ($M = 8.21$, $SD = 0.81$) were recruited through the Royal Holloway, University of London, child database and through opportunistic sampling methods. Written consent was obtained from parents/legal guardians (see Appendices B.2 & C.2) prior to participation, as well as participants' assent (see Appendices D.2 & E). Parents/legal guardians confirmed their child spoke English as a first language, had no known learning or behavioural difficulties (e.g. ADHD, dyslexia or reading impairments), and had no fruit or fruit juice intolerance.

4.2.2 Background measures

Participants completed a series of background measures during two initial practise sessions: three subtests of the British Ability Scales 3rd edition (BAS: verbal similarities, matrices, pattern construction) as measures of acquired verbal knowledge, verbal and non-verbal reasoning, and spatial visualisation, and the Continuous Performance Task (CPT) as a measure of sustained attention. The York Assessment of Reading for Comprehension (YARC) measured passage reading accuracy, reading rate and reading comprehension (see Appendix G), and the Single Word Reading Task (SWRT) sub-test of the YARC measured word reading accuracy (see Appendix H). Average habitual fruit and vegetable intake was also collected through the completion of three-day food diaries (see Appendix L) completed at baseline, between the acute test session and the chronic test session at two weeks, and between the chronic test session at two weeks and the chronic test session at four weeks. This was to ensure participants were complying with the 24-hour low-flavonoid diet before each test session and to make sure that participants were not altering their diet (for example by eating

greater quantities of high-flavonoid foods) over the four-week period. Raw scores and standard scores for these tasks are shown in Table 4.4.1.

4.2.3 Treatments

An acute-on-chronic between-subjects, randomised, placebo-controlled, single-blind design was applied with participants randomly allocated to receive either the 30 g freeze-dried WBB powder treatment or the sugar and vitamin-C matched placebo (WBB: *n* = 15; placebo: *n* = 15 placebo). For acute testing, treatments were prepared by the researcher immediately prior to consumption. For chronic testing, the dry treatment constituents were prepared and sealed within individual sachets that were stored in the participant's freezer. Parents were instructed to administer the treatment at breakfast each morning, were shown how to prepare the treatment for consumption, and were provided with all the necessary equipment. See Section 2.2.3 for further information on treatment constituents, and Section 4.2.5 for further information on treatment preparation and administration.

4.2.4 Cognitive measures

All measures of cognition, reading behaviour, and mood, used previously (Studies 1 & 2; Whyte et al., 2016) were used for the present study: Rey's Auditory Verbal Learning Task (AVLT; Lezak, 1983; see Appendix I), Modified Attention Network Task (MANT; Fan et al., 2002; Whyte et al., 2017), Test of Word Reading Efficiency (TOWRE-2; Torgesen et al., 2012; see Appendix J), and the Positive and Negative Affect Scale for Children (PANAS-C; Laurent et al., 1999; see Appendix K). See Section 2.2.4 for further information on the cognitive domains and reading behaviour assessed by each task, task presentation and completion, and the outcome measures for each task.

4.2.5 Procedure

Two initial practise sessions were completed at seven and two days prior to baseline testing. Two practise sessions were used instead of one to limit the time children were being tested to reduce effects of cognitive fatigue and boredom on performance. Both practise sessions lasted approximately one hour and involved the completion of the following background measures: BAS, CPT, YARC, SWRT. In addition, participants completed a shortened version of the cognitive test battery: AVLT, MANT, PANAS-C, to reduce practise effects. All participants filled out a three-day food diary to assess habitual diet and flavonoid

intake and followed a 24-hour low-flavonoid diet (see Appendix F) before each test session. Participants were asked to abstain from taking any vitamin/mineral supplements throughout the study period.

4.2.5.1 Acute

To align with the previous acute studies (Studies 1 & 2), participants took part in an acute test day which took place at the weekend to fit around school. Both baseline and the acute test session completed two hours after the initial treatment took place in a quiet room at the participant's home or at the University. Time of day was not controlled for in this instance as it was important for testing to be flexible around family lifestyle; the impact of time of day on the data is addressed in Section 4.5. Here, baseline testing was followed immediately by treatment consumption, with the acute test session taking place two hours later. Participants were only allowed water and avoided exercise during this two-hour period. See Section 2.2.5 for further information on the acute testing procedure.

After the acute test session, participants were supplied with 14 days of their randomised treatment, one bottle of Rocks orange squash, an opaque flask, and opaque straws, along with instructions for parents on treatment preparation and administration (see Appendix M). Participants were asked to consume their treatment daily in the morning and to consume it immediately after mixing. All were provided with a drinks log (see Appendix N) to mark when treatments were consumed to aid compliance. Participants further completed a three-day food diary before the next test session to assess habitual diet and to note any changes over the test period that might influence the results.

4.2.5.2 Chronic

After 14 days of treatment, participants followed a 24-hour low-flavonoid diet; no treatment was consumed over this 24-hour period. This 24-hour abstinence from treatment consumption allowed for the examination of the accumulative effects of WBB intake on cognition, reading behaviour, and mood, at the two-week point, and not the effects from the last treatment consumed. Participants then completed a matched version of the cognitive test battery, incorporating the same tasks and completed in the same order as for the acute test session. Following completion of the cognitive test battery, a further 14 days of treatment was supplied to each participant, along with a three-day food dairy and drinks log.

After consumption of this last batch of treatment and again following a 24-hour low-flavonoid diet and treatment free period, participants again completed a matched version of the cognitive test battery at four weeks. This concluded the study. All participants and parents were debriefed and children were provided with a small reward: a t-shirt.

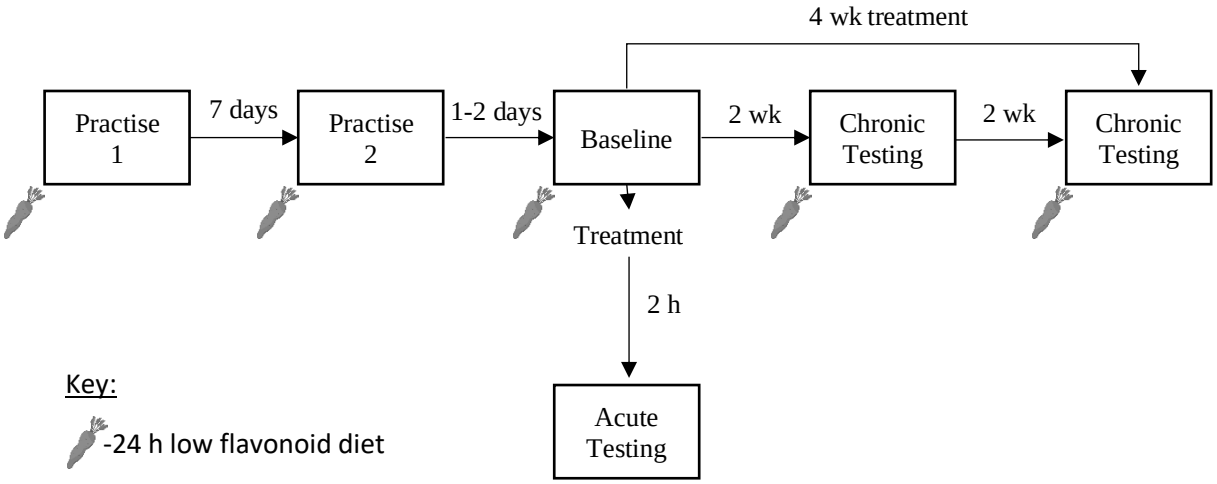


Figure 4.2.5: A diagram to show the acute-on-chronic procedure, including when the 24 h low flavonoid diet was followed.

4.3 Statistical Analysis

4.3.1 Acute

Data were analysed using SPSS (Version 21.0). Differences between treatments (WBB, placebo) for background measures: BAS subtests, CPT omissions and commissions, general reading ability, SWRT, and averaged habitual fruit and vegetable intake per day were examined using independent-samples t-tests. A chi-square was performed to assess sex differences between treatment groups. Means, standard deviations, and group comparisons, are presented in Table 4.4.1.

Linear Mixed-effect Models (LMM) using unstructured covariance matrices were used to analyse data for all dependent variables: all AVLT outcome measures (see Section 2.2.4.1), MANT accuracy and reaction time (ms; correct responses only), both TOWRE-2 SWE and TOWRE-2 PDE, and both PANAS-C PA and NA. The same LMM parameters from Study 1 were employed for the acute arm of the present study (see Section 2.2).

4.3.2 Chronic

Performance over the two chronic test sessions (two weeks and four weeks) was analysed by a separate Linear Mixed-effects Model (LMM) using unstructured covariance matrices to model repeat test sessions. In addition to adopting the LMM parameters outline above in Section 4.3.1, Session (two weeks and four weeks) was included as a repeated factor to assess differences in performance on all dependent variables between the two chronic test sessions. Session was further combined with Treatment, as well as MANT Congruency, Load, and Target Time to assess interactions with performance (Session*Treatment*Congruency, Session*Treatment*Load, Session*Treatment*Target Time). In all models, pairwise comparisons were used where appropriate and Bonferroni corrections were applied to control for type I error. Baseline performance was also included in each model as a fixed factor covariate to control for pre-treatment individual differences only.

4.4 Results

4.4.1 Background measures

The below table (Table 4.4.1) contains the means, standard deviations, ranges, *t* statistics, Cohen's *d,* and *p* values, for the raw scores and standard scores of all background measures, as well as for demographic information such as age and gender.

A significant difference was observed between treatment groups for BAS pattern reconstruction; t(28) = -2.53, $p < .018$, $d = .95$. Here, participants receiving the placebo had greater scores than those receiving WBB. However, as no other differences were observed between the treatment groups for BAS verbal similarities or matrices, the finding suggests that participants with greater spatial visualisation may have been randomly allocated to the placebo group.

No significant differences were observed between treatment groups for: CPT, BAS verbal similarities and matrices, YARC reading accuracy, YARC reading rate, YARC reading comprehension, YARC SWRT, or habitual fruit and vegetable intake. This suggests that both treatment groups were evenly matched in terms of: age, gender, school age group, non-verbal intelligence, sustained attention, reading ability. When considering CPT performance, this cohort achieved average percentage commission errors compared to the norms (commission error mean = 58 – 68% dependent on gender). However, they produced slightly

lower than average percentage omission errors than the norms (omission error mean = 6.5 – 6.7% dependent on gender; Conners et al., 2003). However, it is important to note that the lowest norms provided by the CPT are for children aged nine to eleven years old. Therefore, the slightly lower percentage omission errors would most likely be within the norm for children aged 8 years (the mean age for Study 3). For the BAS, a *t* score of 50 was equivalent to a standard score of 100 (*M* = 100, *SD* = 15). The sample of children performed one standard deviation above the norm for the age group indicating that children had above average ability for: acquired verbal knowledge, verbal and non-verbal reasoning, and spatial visualisation. Further, both groups performed one standard deviation above the norm for their age group for all YARC components: reading accuracy, reading rate, reading comprehension, SWRT. This indicated that all children had average to good reading ability and were matched to the children in Study 1 and Study 2 for reading accuracy, reading rate, and reading comprehension.

Last, children from both treatment groups had an average fruit and vegetable intake of 3 portions per day. This is slightly lower than the national guidelines which states that children of this age should be consuming between four to five portions per day (World Health Organisation, 2006). However, as most children fail to reach this target of four to five daily portions (Department of Health, 2000) and with research indicating much lower portions of fruit and vegetable consumption in general, this sample was thought to have an adequate to good daily habitual intake of fruit and vegetables. This data was used to characterise the sample's habitual flavonoid intake and was not used during the data analyses.

Table 4.4.1: Data for background measures and demographics including raw scores, standard score means, standard deviations, range, *t* statistics, and *p* values

	Placebo (*n* = 15)			**Blueberry (*n* = 15)**				
	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Age	8.23	1.01	7.00-10.05	8.37	0.96	7.00-10.02	-.379	.708
Gender (male:female)^	6:9	---	---	6:9	---	---	---	---
Average fruit/veg intake per day	3.33	1.50	2-6	3.27	1.10	1-5	-.139	.890
Continuous Performance Task								
Omissions (%)	5.33	2.65	1.67-12.08	5.41	2.58	1.67-12.50	.086	.932
Commissions (%)	60	23.64	26.67-90	61.43	19.29	23.33-90	.178	.860

Table 4.4.1: *continued…*

	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
British Ability Scale								
Verbal Similarities								
t-score	53.53	8.16	39-67	52.47	6.74	37-64	-.390	.699
Matrices								
t-score	59.60	12.10	47-80	59.27	11.54	39-77	-.077	.939
Pattern Construction								
t-score	63.07	7.24	51-80	56.07	7.43	42-64	-2.526	.018*
York Assessment of Reading for Comprehension								
Reading Accuracy								
Raw scores	59.13	8.58	37-70	56.93	9.11	42-70	---	---
Standard scores	110.93	7.91	90-124	111.80	1.19	93-125	.245	.808

Table 4.4.1: *continued…*

	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Reading Rate								
Raw scores (ms)	77.20	10.47	59-97	74.27	13.61	59-97	---	---
Standard scores (ms)	115.89	11.59	90-130	116.80	10.51	97-130	.231	.819
Reading Comprehension								
Raw scores	12.47	2.03	9-16	13.07	1.28	10-15	---	---
Standard scores	122.40	7.54	109-130	121.93	6.35	112-130	-.183	.856
Single Word Reading Task								
Raw scores	44.53	8.44	29-55	45.53	5.64	33-53	.381	.706
Standard scores	112.60	10.87	---	113.47	8.51	---	---	---

Notes: York Assessment of Reading for Comprehension standard scores (M = 100, SD = 15).

^ No significant differences were observed between the number of males and females receiving each treatment ($\chi^2 < .001$, $p = .645$).

4.4.2 Acute analysis

4.4.2.1 AVLT

As expected from the previous research (Studies 1 & 2; Whyte & Williams, 2015; Whyte et al., 2016), word recall performance increased between recalls A1-A5. Following the presentation of the distractor list B (recall B), word recall declined. Word recall performance then increased again across recalls A6 and A7 (Figure 4.4.2.1). Means and standard deviations for all AVLT dependent variables are presented in Table 4.4.2.1A.

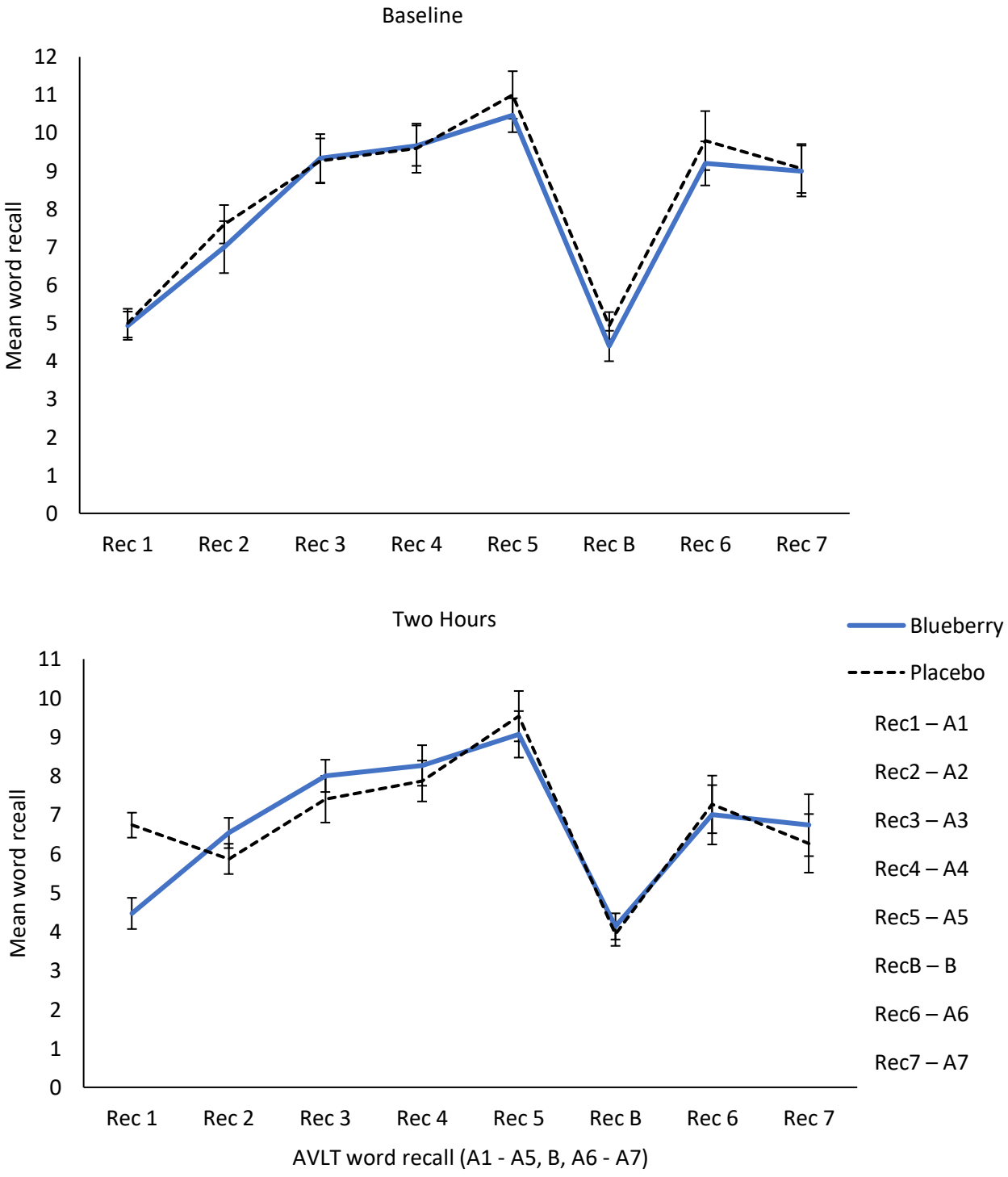


Figure 4.4.2.1A: Mean word recall at each recall point (A1-A5, B, A6-A7) for WBB and placebo at baseline and two hours post-consumption. Adjusted model means and standard error presented.

Table 4.4.2.1A: Means and standard deviations for all AVLT dependent variables at baseline and two hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Word Span	4.93	1.44	5	1.46	.901	5	1.46	4.93	1.44	.797
Words Learnt	5.53	1.77	6	2.54	.563	6	2.54	5.53	1.77	.717
Final Acquisition	10.47	1.73	11	2.42	.493	11	2.42	10.47	1.73	.600
Proactive Interference	.53	1.85	.07	1.03	.400	.07	1.03	.53	1.85	.616
Retroactive Interference	1.27	1.75	1.20	1.89	.921	1.20	1.89	1.27	1.75	.798
Short Delayed Recall	9.2	2.24	9.80	3	.540	9.80	3.01	9.20	2.24	.804
Long Delayed Recall	9	2.59	9.07	2.49	.943	9.07	2.49	9	2.59	.673
Total Delayed Recall	18.20	4.69	18.87	5.24	.716	18.87	5.24	18.20	4.69	.924
Total Recall (A1-A5)	41.4	8.45	42.47	8.74	.737	42.47	8.74	41.40	8.45	.702
Word Recognition	10.93	3.89	11.93	2.49	.410	11.93	2.49	11.71	2.56	.945

The fixed effect of Treatment did not significantly predict performance on any AVLT outcome measure (Table 4.4.2.1B).

Table 4.4.2.1B: β coefficients and *p* values for the fixed effect of treatment for all AVLT LMM analyses at two hours.

Fixed effects	β coefficients	*p* values
Word Span	-.10	.816
Words Learnt	-.18	.837
Final Acquisition	-.13	.859
Proactive Interference	-.41	.522
Retroactive Interference	-.20	.790
Short Delayed Recall	.11	.897
Long Delayed Recall	.52	.467
Total Delayed Recall	.75	.595
Total Recall (A1-A5)	1.81	.257
Word Recognition	2.68	.301

4.4.2.2 MANT

4.4.2.2.1 Accuracy

The present study demonstrated greater MANT accuracy for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials) at two hours, regardless of treatment. This is consistent with that found previously (Studies 1 & 2; Whyte et al., 2016; 2017). The fixed effect of Congruency was a significant predictor of accuracy; F(1,232.64) = 7.01, MSE = .02, p = .009, n^2 = .19; with pairwise comparisons revealing greater accuracy for congruent trials compared to incongruent trials (p = .009). A trend was observed whereby Target Time was a near significant predictor of MANT accuracy; F(1,211.69) = 3.18, MSE = .02, p = .076, n^2

= .01, with pairwise comparisons revealed no significant difference in accuracy. Load did not predict accuracy. In general, accuracy performance was high with participants gaining between 91-93% of correct responses at two hours, suggesting that participants from both treatment groups were performing at ceiling. Means and standard deviations are presented in Table 4.4.2.2.1A.

Table 4.4.2.2.1A: Means and standard deviations for MANT accuracy at baseline and two hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency										
Congruent	.91	.06	.93	.10	.670	.93	.04	.93	.05	.959
Incongruent	.82	.15	.83	.16	.810	.91	.09	.91	.09	.771
Load										
High	.84	.12	.84	.13	.683	.91	.06	.92	.07	.673
Medium	.89	.08	.89	.11	.821	.92	.04	.92	.06	.947
Target Time										
120 ms	.85	.10	.86	.13	.900	.91	.06	.92	.07	.673
500 ms	.88	.10	.89	.11	.589	.93	.05	.92	.07	.976

The fixed effect of Treatment did not significantly predict MANT accuracy performance for any of the MANT parameters. No Treatment x MANT parameter interactions were observed (Table 4.4.2.2.1B).

Table 4.4.2.2.1B: β coefficients and *p* values for the fixed effects for MANT accuracy LMM analysis at two hours.

Fixed effects	**β coefficient**	***p* value**
Treatment	.01	.908
Congruency	.04	.009
Load	-.01	.710
Target Time	-.01	.076
Treatment*Congruency	-.04	.503
Treatment*Load	< -.001	.636
Treatment*Target Time	-.01	.264

4.4.2.2.2 Reaction time (ms; correct responses only)

Consistent with the previous research (Studies 1 & 2; Whyte et al., 2016; 2017). The present study demonstrated faster MANT reaction times for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials) at two hours, regardless of treatment. The fixed effect of Congruency was a significant predictor of reaction time; F(1,228.21) = 25.95, MSE = 12.26, $p < .001$, $n^2 = .48$, with pairwise comparisons revealing faster reaction times for congruent trials compared to incongruent trials. The fixed effect of Load was a further significant predictor of reaction time; F(1,208.34) = 4.53, MSE = 11.98, $p = .034$, $n^2 = .14$, with pairwise comparisons revealing faster reaction times observed for medium load trials compared to high load trials. Target Time did not predict reaction time. Means and standard deviations are presented in Table 4.4.2.2.2A.

Table 4.4.2.2.2A: Means and standard deviations for MANT reaction time (ms; correct responses only) at baseline and two hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency										
Congruent	645.69	123.25	658.53	129.65	.783	628.28	107.34	622.51	112.54	.959
Incongruent	700.71	129.02	691.44	136.24	.850	685.67	100.64	670.51	117.93	.771
Load										
High	684.66	131.16	683.78	138.64	.986	649.33	90.29	646.73	109.95	.672
Medium	661.73	119.26	666.18	126.22	.922	645.83	103.66	635.15	111.17	.947
Target Time										
120 ms	646.72	112.58	645.42	127.86	.977	636.59	84.49	632.93	102.87	.673
500 ms	699.67	145.28	704.54	140.82	.926	677.35	125.49	660.09	132.79	.976

A near significant Treatment x Target Time interaction was observed; F(1,201.13) = 3.10, MSE = 10.93, p = .080, n^2 = .02. However, pairwise comparisons revealed no significant difference between treatments for fast and slow trials, or between trials for WBB and placebo.

The fixed effect of Treatment was not a significant predictor of reaction time, and no interactions were observed between Treatment and MANT Congruency or Load (Table 4.4.2.2.2B).

Table 4.4.2.2.2B: β coefficients and *p* values for the fixed effects MANT reaction time (ms; correct responses only) LMM analysis.

Fixed effects	**β coefficient**	***p* value**
Treatment	-20.82	.507
Congruency	-23.41	< .001
Load	6.29	.034
Target Time	-7.74	.853
Treatment*Congruency	4.62	.713
Treatment*Load	4.53	.715
Treatment*Target Time	10.93	.080

4.4.2.3 PANAS-C

The general pattern of results was consistent with previous research (Studies 1 & 2) demonstrating greater self-reported feelings of positive affect and lower self-reported feelings of negative affect. Means and standard deviations are presented in Table 4.4.2.3A.

Table 4.4.2.3A: Means and standard deviations for positive and negative affect at baseline and two hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Positive affect	50.73	11.66	49.87	8.44	.817	53.80	12.16	52.53	7.85	.737
Negative affect	19.93	5.22	16.27	1.33	.065	18.87	5.32	15.73	.88	.032

The fixed effect of Treatment did not significantly predict positive affect or negative affect in this instance (Table 4.4.2.3.B).

Table 4.4.2.3B: β coefficients and *p* values for the fixed effect of treatment for positive affect and negative affect LMM analysis.

Fixed effects	**β coefficient**	***p* value**
Positive Affect	-.53	.785
Negative Affect	-1.48	.197

4.4.2.4 TOWRE-2

In line with previous research (Studies 1 & 2) and the general pattern of expected performance, participants had greater sight word reading efficiency (SWE) compared to phonemic decoding efficiency (PDE) at two hours, regardless of treatment. This demonstrates that participants were more efficient at reading aloud known words compared

to newly encountered words. Means and standard deviations are presented in Table 4.4.2.4A.

Table 4.4.2.4A: Means and standard deviations for SWE and PDE at baseline and two-hours post-consumption.

	Baseline					**Two hours**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
SWE	69.80	10.52	65.47	11.08	.282	69.40	9.43	66.53	11.21	.455
PDE	34.27	11.52	30.87	10.78	.411	34.20	10.14	30.73	13.23	.427

The fixed effect of Treatment was not a predictor of SWE or PDE on this occasion (Table 4.4.2.4B).

Table 4.4.2.4B: β coefficients and *p* values for the fixed effect of treatment for SWE and PDE LMM analysis.

Fixed effects	**β coefficient**	***p* value**
SWE	.94	.535
PDE	-.27	.888

4.4.3 Chronic analysis

4.4.3.1 AVLT

The same general pattern of performance as described in Section 4.4.2.1 was exhibited at both at the two-week and four-week test sessions (Figure 4.4.3.1): an increase in word recall across recalls A1-A5, a sharp decline in word recall for List B, followed by an increase again for recalls A6 and A7 (although word recall performance on these recalls was less than that exhibited for recalls A1-A5). Means and standard deviations are presented in Table 4.4.3.1A.

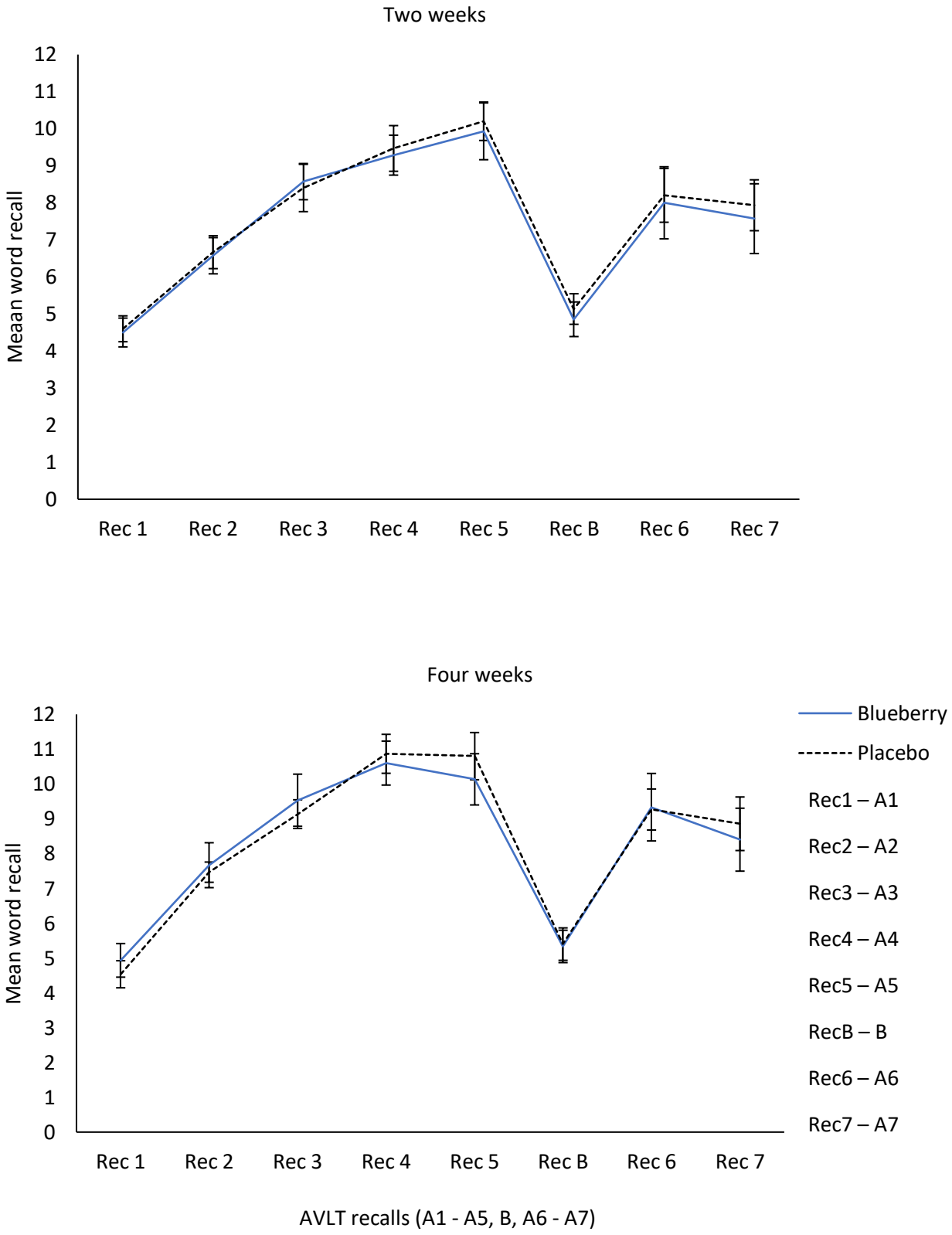


Figure 4.4.3.1: Mean word recall at each recall point (A1-A5, B, A6-A7) for WBB and placebo at two weeks and four weeks. Adjusted model means and standard error presented.

Table 4.4.3.1A: Means and standard deviations for all AVLT dependent measures at baseline, two weeks and four weeks.

	Baseline					**Two weeks**					**Four weeks**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Word Span	4.93	1.44	5	1.46	.901	4.60	1.35	4.20	1.82	.481	4.53	1.51	4.93	1.87	.452
Words Learnt	5.53	1.77	6	2.54	.563	5.60	2.09	5.07	2.79	.593	6.27	2.63	5.20	2.46	.298
Final Acquisition	10.47	1.73	11	2.42	.493	10.20	2.01	9.27	3.77	.515	10.80	2.62	10.13	2.85	.710
Proactive Interference	.53	1.85	.07	1.03	.400	-.53	1.55	-.33	1.59	.652	-.87	2.39	-.40	2.35	.538
Retroactive Interference	1.27	1.75	1.20	1.89	.921	2	1.25	1.80	1.82	.706	1.53	2.20	.80	1.66	.291
Short Delayed Recall	9.2	2.24	9.80	3	.540	8.20	2.81	7.47	4.07	.710	9.27	2.28	9.33	3.75	.706

Table 4.4.3.1A: *continued…*

	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Long Delayed Recall	9	2.59	9.07	2.49	.943	7.93	2.66	7.07	3.92	.434	8.27	3.59	8.40	3.50	.882
Total Delayed Recall	18.20	4.69	18.87	5.24	.716	16.13	5.36	14.53	7.68	.556	17.53	5.29	17.73	7.14	.771
Total Recall (A1-A5)	41.4	8.45	42.47	8.74	.737	39.33	8.40	36.27	13	.508	42.80	5.76	42.87	11.16	.775
Word Recognition	10.93	3.89	11.93	2.49	.410	11.33	3.75	11.27	2.79	.601	10.80	2.98	11.27	3.08	.250

The fixed effect of Session was a significant predictor of performance when assessed independently for: short delayed word recall; F(1,30) = 6.15, MSE = .84, p = .019, n^2 = .18, total delayed word recall; F(1,30) = 4.89, MSE = 1.47, p = .035, n^2 = .15, and total word recall; F(1,30) = 7.31, MSE = 2.63, p = .011, n^2 = .21. All measures indicated greater word recall performance at four weeks compared to two weeks, regardless of treatment (Figure 4.4.3.1B). This increase in performance between sessions is likely indicative of practise effects as treatment did not influence performance.

The fixed effect of Treatment was not a significant predictor of any AVLT outcome measure and no subsequent Treatment x Session interactions were observed.

Table 4.4.3.1B: β coefficients and *p* values for the fixed effects of Treatment and Session for all AVLT LMM analyses over the four-week period.

	β coefficient	*p* values
Word Span		
Treatment	.43	.948
Session	.07	.325
Treatment* Session	-.80	.239
Words Learnt		
Treatment	-.99	.329
Session	-.67	.434
Treatment*Session	.53	.601
Final Acquisition		
Treatment	-.34	.528
Session	-.60	.180
Treatment*Session	-.27	.805
Proactive Interference		
Treatment	.52	.417
Session	.33	.709
Treatment*Session	-.27	.804

Table 4.4.3.1B: *continued…*

	β coefficient	*p* values
Retroactive Interference		
Treatment	-.74	.249
Session	.47	.130
Treatment*Session	.53	.576
Short Delayed Recall		
Treatment	.38	.985
Session	-1.07	.019
Treatment*Session	-.80	.504
Long Delayed Recall		
Treatment	.17	.730
Session	-.33	.153
Treatment*Session	-1	.386
Total Delayed Recall		
Treatment	.59	.864
Session	-1.40	.035
Treatment*Session	-1.80	.394
Total Word Recall		
Treatment	.71	.730
Session	-3.47	.011
Treatment*Session	-3.13	.407
Word Recognition		
Treatment	1	.311
Session	.53	.597
Treatment*Session	-.53	.597

4.4.3.2 MANT

4.4.3.2.1 Accuracy

As expected from previous research (Whyte et al., 2016; 2017; Studies 1 & 2), MANT accuracy was greater for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials). The fixed effect of Congruency was a significant predictor of MANT accuracy performance; F(1,210.25) = 45.48, MSE = .01, $p < .001$, $n^2 = .43$, with pairwise comparisons revealing greater accuracy for congruent trials compared to incongruent trials. Similarly, the fixed effect of Target Time was a further significant predictor of MANT accuracy performance; F(1,210.25) = 13.13, MSE = -.02, $p < .001$, $n^2 = .25$, with pairwise comparisons revealing greater accuracy for slow trials compared to fast trials. Load was also a significant predictor of MANT accuracy; F(1,210.25) = 4.79, MSE = .01, $p = .030$, $n^2 = .15$, with pairwise comparisons revealing greater accuracy for medium load trials compared to high load trials. In general, accuracy performance was high with participants gaining between 93-97% of correct responses at the four-week test session suggesting that participants from both treatment groups were performing at ceiling. Means and standard deviations are presented in Table 4.4.3.2.1A.

Table 4.4.3.2.1A: Means and standard deviations for MANT accuracy at baseline, two weeks and four weeks.

	Baseline					**Two weeks**					**Four weeks**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency															
Congruent	.91	.06	.93	.10	.670	.97	.02	.96	.04	.502	.97	.02	.96	.04	.125
Incongruent	.82	.15	.83	.16	.810	.93	.05	.94	.08	.805	.92	.07	.93	.06	793
Load															
High	.84	.12	.84	.13	.683	.94	.04	.95	.05	.565	.94	.05	.94	.05	.774
Medium	.89	.08	.89	.11	.821	.96	.03	.95	.06	.474	.96	.03	.94	.05	.269
Target Time															
120 ms	.85	.10	.86	.13	.900	.94	.03	.93	.06	.795	.94	.04	.93	.06	.578
500 ms	.88	.10	.89	.11	.589	.96	.03	.96	.05	.837	.96	.05	.96	.05	.919

A significant Treatment x Load interaction was observed; F(1,210.25) = 4.79, MSE = .01, p = 030, n^2 = .21, with pairwise comparisons revealing a significant difference between high load and medium load trials for placebo only (p < .005). Here, greater accuracy was observed for the medium load trials compared to the high load trials, regardless of session. This suggests that participants in the placebo group had greater accuracy for the less cognitively demanding medium load trials. No difference was observed between trials for WBB, or between treatment groups.

Further, a near significant Treatment x Congruency interaction was observed; F(1,210.25) = 3.45, MSE = .01, p = .065, n^2 = .23, with pairwise comparisons revealing a significant difference between congruent and incongruent trials for placebo (p < .001) and WBB (p < .001). Here, greater accuracy was observed for the congruent trials compared to the incongruent trials, regardless of session. This suggests that participants in general had significant greater accuracy for the less cognitively demanding trials. No differences were observed between treatments for congruent or incongruent trials.

The fixed effect of Session was not significant predictors of MANT accuracy when assessed independently, and there were no further interactions between fixed effects and MANT parameters (Table 4.4.3.2.1B).

Table 4.4.3.2.1B: β coefficients and *p* values for the fixed effects for MANT accuracy LMM analysis over the four-week period.

Fixed effects	**β coefficient**	***p* value**
Treatment	< -.001	.811
Session	.01	.276
Congruency	.05	< .001
Load	-.02	.030
Target Time	-.01	< .001
Treatment*Session	< -.001	.507
Treatment*Congruency	-.02	.065
Treatment*Load	.02	.030
Treatment*Target Time	-.01	.405
Treatment*Session*Congruency	< -.005	.591
Treatment*Session*Load	< .005	.916
Treatment*Session*Target Time	< .001	.985

4.4.3.2.2 Reaction time for correct responses only (ms)

Consistent with the previous research from Whyte et al. (2016; 2017) and Studies 1 and 2, MANT reaction times for correct responses only were faster for the less cognitively demanding trials (congruent, medium load, slow trials) compared to the more cognitively demanding trials (incongruent, high load, fast trials). The fixed effect of Congruency was a significant predictor of MANT reaction times; F(1,197.99) = 40.51 MSE = 9.26, $p < .001$, $n^2 = .26$, with pairwise comparisons revealing faster reaction times for congruent trials compared to the incongruent trials. The fixed effect of Target Time was also a significant predictor of MANT reaction times; F(1,197.99) = 77.87, MSE = 9.26, $p < .001$, $n^2 = .34$, with pairwise comparisons revealing faster reaction times for fast trials at 120 ms compared to slow trials

at 500 ms. Load also predicted MANT reaction times; F(1,197.99) = .02, MSE = 9.26, p = .008, n^2 = < .001, with pairwise comparisons revealing faster reaction times for medium load trials compared to high load trials. Means and standard deviations are presented in Table 4.4.3.2.2A.

Table 4.4.3.2.2A: Means and standard deviations for MANT reaction times for correct responses only (ms) at baseline, two weeks and four weeks.

	Baseline					**Two weeks**					**Four weeks**				
	Placebo (n = 15)		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**			**Placebo (n = 15)**		**WBB (n = 15)**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Congruency															
Congruent	645.69	123.25	658.53	129.65	.783	603.91	97.92	620.39	98.21	.649	593.25	104.41	616.26	96.35	.536
Incongruent	700.71	129.02	691.44	136.24	.850	646.85	93.88	652.88	96.46	.864	627.76	95.60	632.43	95.60	.892
Load															
High	684.66	131.16	683.78	138.64	.986	632.98	97.29	646.35	100.95	.715	615.64	100.66	628.57	93.49	.718
Medium	661.73	119.26	666.18	126.22	.922	617.78	92.85	626.92	93.21	.790	605.37	98.32	620.12	92.89	.676

Target Time															
120 ms	646.72	112.58	645.42	127.86	.977	609.98	85.67	613.43	83.99	.912	594.99	83.93	591.04	77.73	.895
500 ms	699.67	145.28	704.54	140.82	.926	640.78	108.07	659.84	118.31	.649	626.02	118.16	657.65	121.09	.475

Although the fixed effect of Treatment was not a significant predictor of MANT reaction time, a significant Treatment x Session x Target Time interaction was observed ($p < .032$; Figure 4.4.3.2.2). Planned comparisons revealed a significant difference between Sessions for fast trials for those consuming WBB ($p < .002$) whereby reaction times were significantly faster at four weeks compared to two weeks. Similarly, for placebo, a significant difference between Sessions for both fast trials ($p = .039$) and slow trials ($p = .042$) was observed with significantly faster reaction times at four weeks compared to two weeks. There were no significant differences between treatment groups at two weeks or four weeks for either the fast or slow trials.

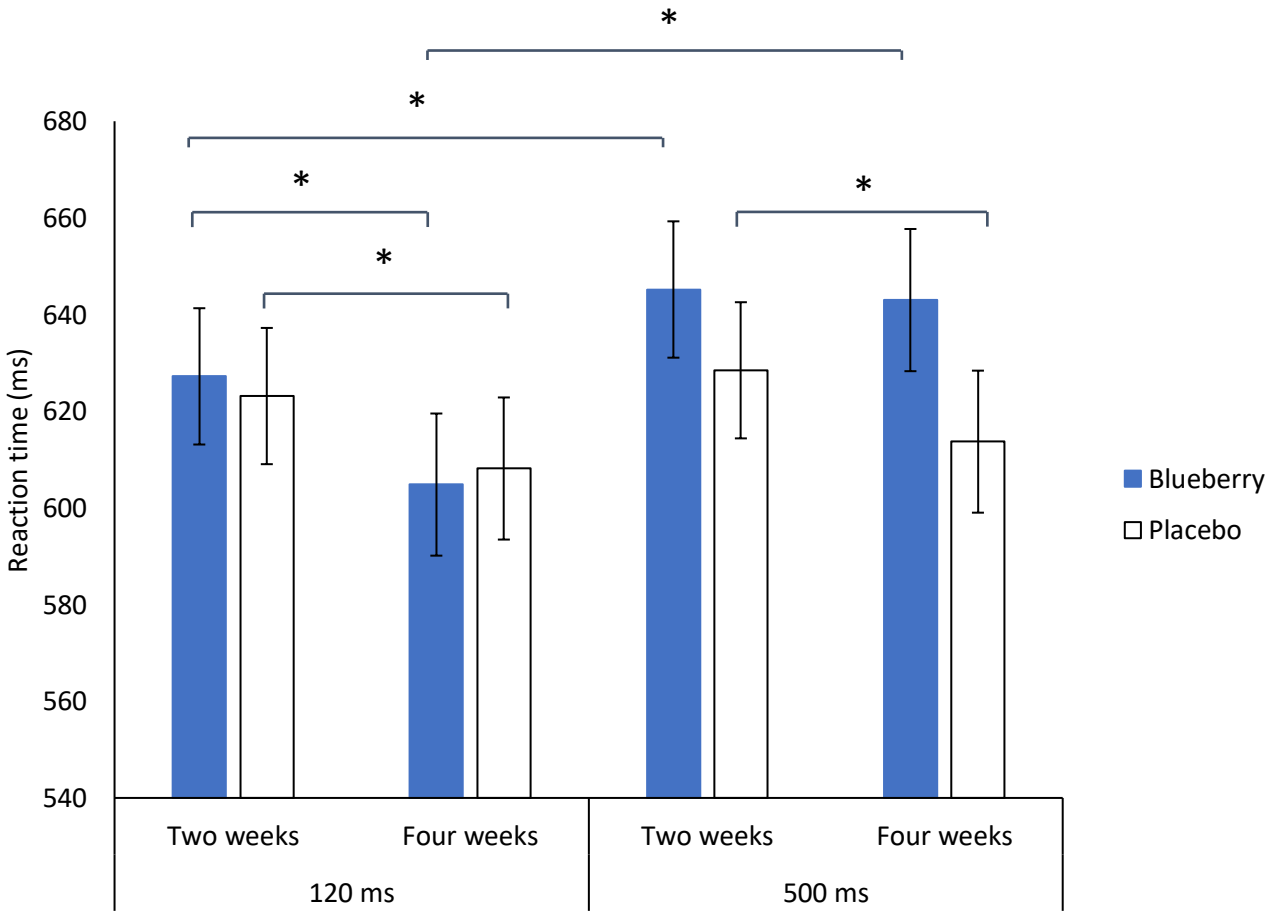


Figure 4.4.3.2.2: The significant Treatment*Session*Target Time interaction for MANT reaction time ($p = .032$) observed a number of significant differences. For fast trials, both WBB and placebo showed a significant difference in reaction times between two weeks and four weeks. Here faster reaction times were observed at four weeks compared to two weeks for both treatment groups (WBB: $p < .005$, placebo: $p = .039$). For placebo only, there was a significant difference between two weeks and four weeks for slow trials ($p = .042$) with

faster reaction times at four weeks compared to two weeks. Last, only WBB showed a significant difference between fast and slow trials at both two weeks (p = .016) and four weeks (p < .001). In general, faster reaction times were observed for the fast trials. No difference between fast and slow trials was observed for placebo. No differences were observed between treatment groups at two weeks or four weeks for either fast or slow trials. Adjusted model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.

The fixed effect of Session was a significant predictor of MANT reaction time; F(1,240) = 14.17, MSE = 10.21, p < .001, n^2 = .29, with pairwise comparisons revealing faster responses at four weeks compared to two weeks, regardless of treatment (Table 4.4.3.2.2B).

The fixed effect of Treatment did not predict MANT reaction time for correct responses only (ms) and no further interactions were observed.

Table 4.4.3.2.2B: β coefficients and *p* values for the fixed effects for MANT reaction time for correct responses only (ms) LMM analysis over the four-week period.

Fixed effects	**β coefficient**	***p* value**
Treatment	23.37	.704
Session	16.52	< .001
Congruency	-34.51	< .001
Load	10.27	.008
Target Time	-31.02	< .001
Treatment*Session	-11.65	.744
Treatment x Congruency	18.35	.148
Treatment x Load	-1.82	.903
Treatment x Target Time	-35.58	.010
Treatment x Session x Congruency	-16.33	.200
Treatment x Session x Load	10.97	.500
Treatment x Session x Target Time	-12.42	.032

4.4.3.3 PANAS-C

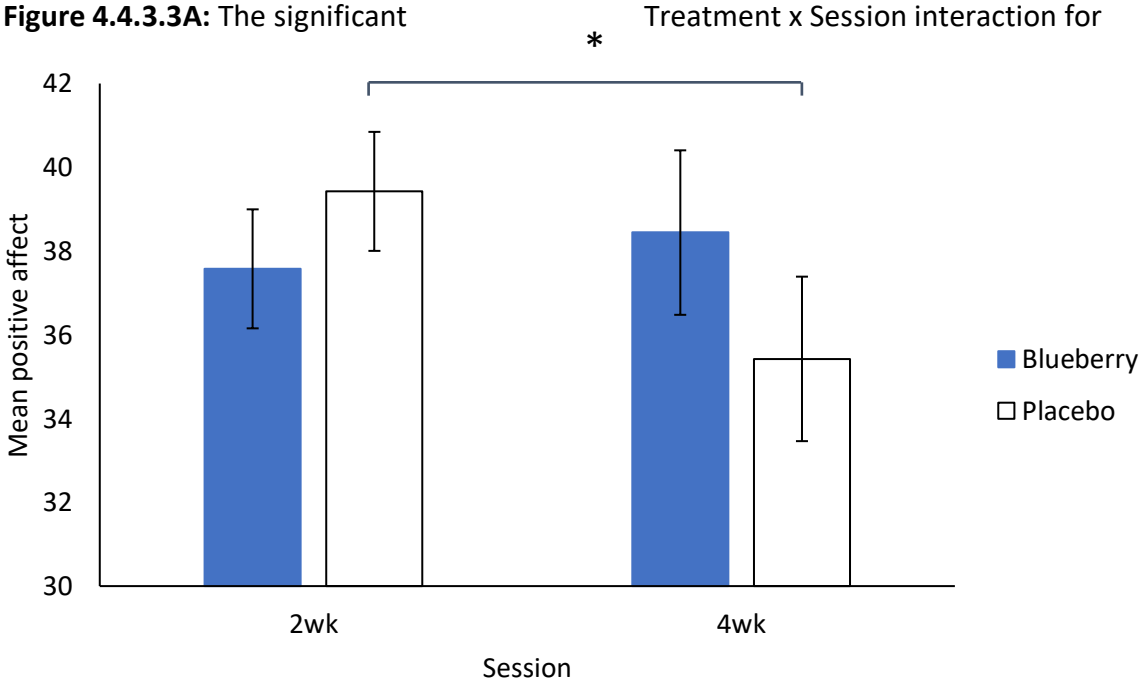
The present findings match those found previously (Studies 1 & 2) where participants self-reported greater feelings of positive affect whilst self-reporting lower feelings of negative affect. Means and standard deviations are presented in Table 4.4.3.3A.

Table 4.4.3.3A: Means and standard deviations for positive and negative affect at baseline, two weeks and four weeks.

	Baseline					**Two weeks**					**Four Weeks**				
	Placebo		**WBB**			**Placebo**		**WBB**			**Placebo**		**WBB**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
Positive affect	50.73	11.66	49.87	8.44	.817	49.53	11.95	47.07	9.25	.532	44.60	14.64	49.40	8.47	.281
Negative affect	19.93	5.22	16.27	1.33	.065	17.73	5.19	16.40	1.88	.357	22.20	10.94	16.40	1.68	.052

Although the fixed effects of Treatment and Session did not predict positive affect when analysed independently, there was a significant Treatment x Session interaction; F(1,30) = 9.35, MSE = 2.38, p = .005, n^2 = .25 (Figure 4.4.3.3A). Pairwise comparisons revealed a significant difference in positive affect following placebo (p = .006) between two weeks and four weeks. Specifically, positive affect was lower at four weeks compared to two weeks following chronic placebo consumption. Positive affect remained constant between two weeks and four weeks following WBB consumption. There were no significant differences between treatment groups at each time point

Figure 4.4.3.3A: The significant Treatment x Session interaction for positive affect (p = .005) with pairwise comparisons revealing a significant difference in positive affect between two weeks and four weeks for placebo (p = .006). Here positive affect was lower at four weeks compared to two weeks. No difference was observed for positive affect following WBB consumption and no differences were observed between treatment groups at each time point. Adjusted model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.



The fixed effect of Treatment was not a significant predictor of negative affect, however a significant Treatment x Session interaction was further observed; F(1,30) = 4.81, MSE = 2.04, p = .036, n^2 = .15 (Figure 4.4.3.3B). Pairwise comparisons revealed a significant increase in negative affect at four weeks compared to two weeks for placebo consumption only (p < .005). Negative affect remained constant between two weeks and four weeks following WBB. No differences were observed between treatments groups at each time point.

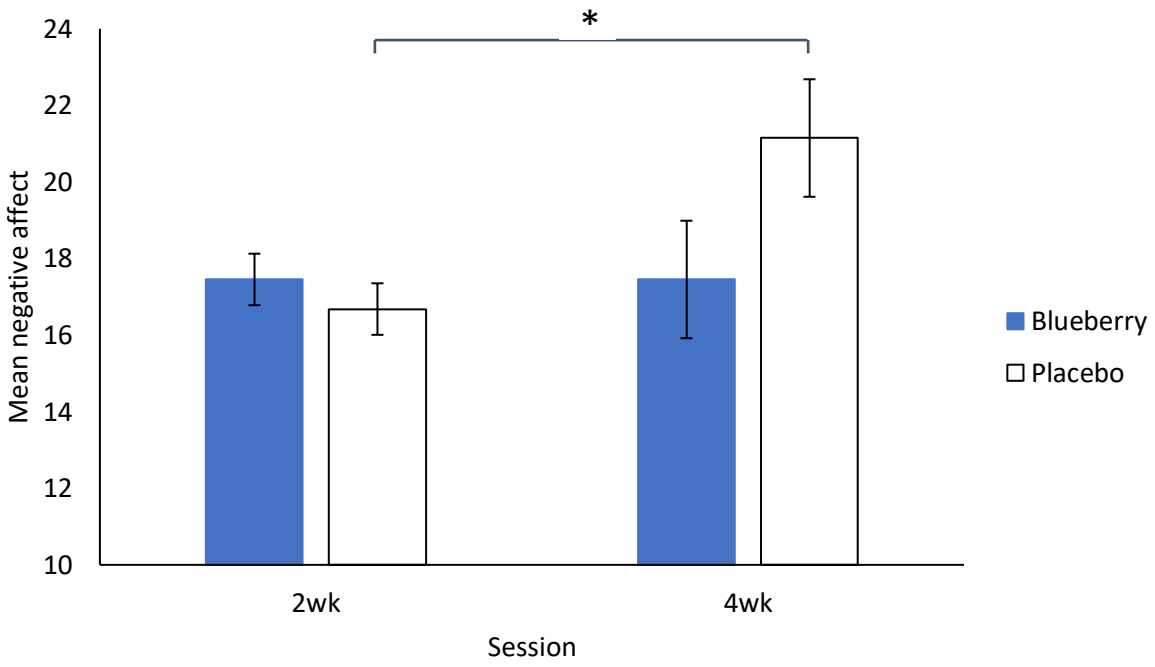


Figure 4.4.3.3B: The significant Treatment x Session interaction for negative affect (p = .036) with pairwise comparisons revealing a significant difference in negative affect between two weeks and four weeks for placebo (p < .004). Here, greater negative affect was observed at four weeks compared to two weeks. No difference was observed for negative affect following WBB consumption and no differences were observed between treatment groups at each time point. Adjusted model means and standard error presented, adjusted for baseline performance. LMM with baseline as a covariate.

Further, the fixed effect of Session significantly predicted negative affect; F(1,30) = 4.81, MSE = 1.44, p = .036, n^2 = .15, with pairwise comparisons revealing an increase in self-reported affect from two weeks to four weeks, regardless of treatment (Table 4.4.3.3B).

Table 4.4.3.3B: β coefficients and *p* values for the fixed effects for positive affect and negative affect LMM analyses over the four-week period.

Fixed effects	**β coefficient**	***p* value**
Positive Affect		
Treatment	5.45	.518
Session	4.93	.283
Treatment*Session	-7.27	.005
Negative Affect		
Treatment	-3.69	.285
Session	-4.47	.036
Treatment*Session	4.47	.036

4.4.3.4 TOWRE-2

Consistent with the previous findings, and following the general expected pattern of performance, participants had greater SWE performance compared to PDE performance over the four-week period. Means and standard deviations are presented in Table 4.4.3.3A.

Table 4.4.3.3A: Means and standard deviations for SWE and PDE at baseline, two weeks and four weeks.

	Baseline					**Two weeks**					**Four weeks**				
	Placebo		**WBB**			**Placebo**		**WBB**			**Placebo**		**WBB**		
	M	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***	***M***	***SD***	***M***	***SD***	***p value***
SWE	69.80	10.52	65.47	11.08	.282	68.93	10.51	66.73	11.05	.581	67.33	8.10	68.13	10.88	.821
PDE	34.27	11.52	30.87	10.78	.411	35.60	10.93	33.13	13.57	.588	36.47	10.69	33.20	12.92	.457

The fixed effects of Treatment and Session were not significant predictors of SWE, and no significant Treatment x Session interaction was observed.

The fixed effects of Treatment and Session were not significant predictors of PDE, and no significant Treatment x Session interaction was observed (Table 4.4.3.3B).

Table 4.4.3.3B: β coefficients and *p* values for both SWE and PDE LMM analyses.

Fixed effects	**β coefficient**	***p* value**
SWE		
Treatment	3.18	.290
Session	1.60	.930
Treatment*Session	-3.00	.192
PDE		
Treatment	.03	.761
Session	-.87	.533
Treatment*Session	.80	.593

4.5 Discussion

The present study aimed to examine the acute and chronic effects of a daily 30 g freeze-dried WBB powder treatment consumed over a four-week period on the cognition, reading behaviour, and mood, of seven to ten-year olds. This was an acute-on-chronic design not only examining the acute effects of WBB intake two hours after an initial treatment (in line with Studies 1 & 2), but also the chronic effects of daily WBB consumption over four weeks; a novel investigation in this population. The acute arm of this study observed no effects of either treatment group on cognition, reading behaviour, or mood. In contrast, the chronic arm of this study observed a number of treatment effects and interactions for cognition and mood. No chronic effects were observed for reading behaviour.

The chronic arm of this study observed a significant Treatment x Session x Target Time interaction for executive function reaction time (ms; correct responses only). For the more cognitively demanding fast trials, both treatment groups showed a significant difference in reaction times between two weeks and four weeks. Specifically, both treatment groups had significantly faster reaction times at four weeks compared to two weeks. For the less

cognitively demanding slow trials, only the placebo demonstrated a significant difference between two weeks and four weeks. Again, faster reaction times were observed at four weeks compared to two weeks. Executive function reaction times for these less cognitively demanding slow trials were maintained over the four-week period following WBB consumption. This difference suggests that the placebo group had faster reaction times in general at four weeks. However, those consuming the WBB treatment only showed significantly faster reaction times for the more cognitively demanding fast trials at four weeks compared to weeks. It is important to note that no significant differences were observed between the treatment groups at either session, suggesting that this interaction was driven by the differences reported between target time trials and not differences between treatment groups. Further, WBB demonstrated a significant difference in executive function reaction times between the fast and slow trials at both two weeks and four weeks.

For WBB only, significant differences in reaction times were observed between the fast and slow trials. Here, participants had faster reaction times on the fast trials and slower reaction times on the slow trials; a finding observed at two weeks and four weeks. This suggests a potential pacing effect whereby participants receiving the WBB treatment were alternating their response rate dependent on the rate at which the stimuli were presented. For example, participants responded to stimuli at a faster rate for the fast trials, whilst responding at a slower rate for the slow trials. This pattern of reaction time performance was not observed following the placebo where no significant differences in reaction times were observed between the fast and slow trials. This suggests that chronic WBB helps moderate response rates to executive function tasks of differing degrees of difficulty. However, this pacing effect did not influence executive function accuracy performance.

Further, the chronic arm of this study observed that both treatment groups had significantly greater executive function accuracy for the less cognitively demanding congruent trials, regardless of session. Significantly greater accuracy was also observed for the less cognitively demanding medium load trials but only following placebo, regardless of session. This finding suggests that children in general had greater accuracy for trials which required less cognitive demand. This pattern of performance was expected and has been consistently observed by the previous research (Studies 1 & 2; Whyte et al., 2016; Whyte et al., 2017). No significant differences were observed between the two treatment groups and no effect of chronic WBB was observed for the more cognitively demanding executive

function trials. This is in contrast to the reported executive function reaction time findings. One explanation for this lack of findings is that children from both treatment groups were performing at ceiling for this task, with the MANT accuracy means (Table 4.4.3.2.1A) showing that children achieved between 92-97% correct responses. Further, this effect of ceiling was also reported for the acute data whereby participants from both treatment groups were achieving between 91-93% of correct responses at two hours. Therefore, it may be unrealistic to expect WBB consumption to benefit performance on a cognitive measure where children are already performing extremely well on.

Next, performance for episodic memory short delayed word recall, total delayed word recall, and total word recall, was greater at four weeks compared to two weeks, regardless of treatment. This was indicative of a practice effect as treatment was not a significant predictor of performance for these measures of episodic memory. This lack of acute and chronic findings for word list recall, was not expected and contrasts not only to the hypotheses set out by the present study, but also to the previous acute studies where acute WBB supplementation significantly improved total word recall and short delayed word recall (Studies 1 & 2). The absence of acute WBB effects on AVLT performance at present remains unclear, however the small sample size and subsequent lack of power may have instigated type I error. This may have led to the masking any potential effects of acute and chronic WBB on performance, where further effects may have been significant if adequate power was achieved; for example, the differences in executive function reaction times between treatment groups following acute intake may have reached significance. Indeed, the previous studies recruited a larger cohort of children with a total N = 54 (Study 1) and N = 61 (Study 2), almost double the sample size recruited here. One of several possible explanations for the lack of acute findings was the small sample size recruited. An initial *a priori* power analysis based on previous acute AVLT word span data (Study 1) revealed a total of 42 participants would be needed to achieve the appropriate level of power, with 21 participants per treatment group. However, due to difficulties with recruitment, only 30 participants were recruited with 15 per treatment group; a substantial decrease from that required. Therefore, this indicates that the study was underpowered and highlights the potential masking of both acute and chronic WBB effects on cognition, reading behaviour, and mood. A post-hoc power calculation (using G Power 3.1) based on the word span data collected at baseline (Study 3) indicated that an effect size of d = .048 was achieved by this study, a value

substantially lower than the *d* = .08 aimed for. This highlighted that the study was severely underpowered. Further, the post-hoc power calculation revealed a total of 90 children would need to be recruited for the study to reach adequate power when considering the achieved effect size (*d* = .048). In order to examine the effects of chronic WBB supplementation on these domains of interest, it would be important for future research to recruit a larger sample size to obtain a sufficient level of power to potentially observe the full effects of chronic WBB supplementation on performance. Future research should also consider the use of a cross-over, within-subjects design when testing performance over multiple test sessions. The within-subjects design has greater statistical power than the between-subjects design as the error variance is reduced. Consequently, the within-subjects design can accommodate a smaller sample size whilst retaining greater statistical power. However, effects of practise become more apparent as participants are expected to complete the same cognitive test battery on multiple occasions. In this case, a between-subjects design was employed to minimise the duration of the study. The recruitment phase of this study highlighted that parents were not willing to partake in the study due to the duration. Therefore, a between-subjects design employing a one-month supplementation period was chosen for this study.

Last, the chronic arm of this study observed significant effects of placebo on mood over the four-week period. The findings demonstrated a reduction in positive affect at four weeks compared to two weeks, as well as an increase in negative affect over the four-week period. No significant differences were observed for WBB suggesting a maintenance of both positive affect and negative affect over the four-week period. This finding contrasts to the previous research (Studies 1 & 2) where acute WBB significantly improved positive affect two-hours after intake. As for verbal episodic memory, the small sample size may have contributed to the lack of WBB-related mood effects observed here. At present, it is unclear from these results whether the significant interactions between treatment and session can be explained by the differences observed for placebo, or by the maintenance seen for both positive and negative affect following WBB supplementation. Further, it is important to consider other extraneous variables which may have contributed to the differences in mood observed over the four-week period. Relationships with family, friends, teachers, and other events may have influenced participant's mood, aside from the potential effects of the treatments consumed for this study.

The present study demonstrated that the chronic WBB treatment did not affect reading behaviour as no differences were observed between the treatment groups for either SWE or PDE over the four-week period. At present, this is in line with the acute research where acute WBB intake did not impact reading behaviour (Studies 1 & 2), although this contrasts to the previous PUFAs literature where chronic supplementation significantly improved decoding speed in healthy children (Johnson et al., 2017). The previous studies (Section 2.5; 3.5) both highlighted the need to examine the chronic effects of WBB supplementation on reading behaviour. Specifically, to assess whether the accumulation of berry-flavonoids could aid sight word reading and phonemic decoding efficiency as an acute, single dose of WBB was found to have a no effect. However, in this instance, chronic WBB consumption failed to improve reading behaviour. Again, the small sample size may have impacted the results, masking any effects of chronic WBB supplementation on reading behaviour. In addition, as mentioned previously (Section 2.5; 3.5), the reading measures used may not be sensitive enough to measure the effect of berry-flavonoid interventions on reading in children. The TOWRE-2 ultimately measures the number of words and non-words read aloud correctly in a limited period of time, thus assessing the fluency and accuracy of reading aloud. This timed element may stop participants from producing the correct pronunciation for a word/non-word in favour of reading as many words/non-words as possible within the 45 seconds, regardless of whether the pronunciation is correct or not. As no effects of either acute or chronic WBB supplementation were observed for the automaticity of sight word reading or phonemic decoding efficiency, future research should focus on an aspect of reading which is similar to that assessed by the AVLT where acute WBB intake improved list-wise word learning via word list recall (Studies 1 & 2; Whyte & Williams, 2015; Whyte et al., 2016). With this in mind, one hypothesis to consider is whether berry-flavonoid interventions can improve learning to read words. Study 4 will examine whether an acute WBB treatment can improve children's ability to learn to read words when presented in a story context, thus mimicking the natural process by which children learn new words when reading, and whether the acute WBB treatment can aid the learning of novel orthography and phonology. Last, participants from both treatment groups had above average reading ability when assessed by the YARC at baseline. Here, participants performed one standard deviation above their mean age norms for all three components of the YARC: reading accuracy, reading rate, and reading comprehension. Therefore, as the sample were already

performing above their age range, it may be unrealistic to expect either acute or chronic WBB supplementation to have an impact on their reading ability. Instead, chronic WBB supplementation may exert a benefit to reading behaviour in children who are underperforming for their age, such as observed by the previous PUFA literature (Richardson et al., 2012); another avenue for future research examining the effects of chronic WBB supplementation on reading behaviour in children.

Aside from the small sample size recruited, several other methodological issues arose from this study which should be addressed for future replication. For this study alone, opportunistic sampling methods were used to recruit participants, primarily through word of mouth and through a research database established by the Department of Psychology at Royal Holloway, University of London. Here, opt-in recruitment occurred whereby parents contacted the researcher if they were interested in their child participating in the study. Therefore, it was highly likely that these parents had a vested interest in this area of research and were highly motivated for their child to take part.

Further, the testing environments used should be considered as these were not consistent between test sessions or amongst participants. The previous acute studies (Studies 1 & 2) were conducted in controlled, quiet learning environments at the participants' schools. In comparison, the present study used a mixture of differing testing environments, testing participants at home or at the University in order to be flexible around busy family lifestyles. Differences in motivation and distraction levels at the different testing environments could have resulted in impaired attention to the cognitive test battery. For example, when tested at home participants may have been distracted or reluctant to complete the cognitive test battery as they associate play and relaxation with the home environment. This is in contrast to the educational set up at the University where participants were subjected to fewer distractions and hence could sustain greater attention to the cognitive test battery. Future research should consider the use of a standardised testing environment where all participants are tested in the same location for all test sessions, controlled for noise and distractions which may affect performance.

As demonstrated by Study 2, time of day significantly interacted with the effects of acute WBB supplementation on cognition, and suggested another potential explanation for the lack of acute WBB effects observed in the present study. In this instance, time of day was not

recorded or controlled for as its effects on the impact of acute WBB consumption on cognition were unknown at the time of data collection for the present study; Studies 2 and 3 were conducted at the same time. Therefore, acute testing took place both in the morning and in the afternoon. Merging data from participants tested across the day could have masked the effect of acute WBB supplementation on cognitive performance, thus resulting in the lack of acute findings observed here. Indeed, Study 2 highlighted greater effects of acute WBB intake on performance when children were tested in the afternoon. Therefore, if the present study tested the majority of children in the morning on the acute test day, these findings may be masking any potential WBB effects for those tested in the afternoon. However, as time of day was not recorded for the present study, this explanation for the potential absence of effects must be considered with caution.

Next, participants recruited for the present study had an average fruit and vegetable intake of three portions per day. Although this is slightly lower than the national guidelines, which recommend an intake of four to five portions per day for children of this age (World Health Organisation, 2006), most children fail to reach this target of four to five daily portions (Department of Health, 2000) with research indicating much lower portions of fruit and vegetable consumption in general. Indeed, Upton et al. (2012) demonstrated that only 2% of four to eleven-year olds consumed one or more portions of fruit and 5% consumed one or more portions of vegetables per day. Therefore, as the participants recruited for the present study already had a good daily intake of fruits and vegetables, it is important to consider whether additional flavonoid supplementation beyond that gained from their habitual diet would benefit cognition. Future research should consider the effect of berry-flavonoid supplementation in those with a low habitual fruit and vegetable intake, and whether additional consumption can aid cognition, reading behaviour, and mood.

In line with the above average reading ability reported at baseline for this sample, participants from both treatment groups also demonstrated above average performance on the measures of sustained attention, verbal and non-verbal intelligence. As stated above for reading behaviour, chronic WBB supplementation may not benefit children already performing within or above their age norms. Instead, chronic benefits of WBB may be observed in children who have poor sustained attention and verbal/non-verbal intelligence for their age range. Indeed, the most consistent benefits of chronic PUFAs on reading ability were observed in underperforming children (Richardson et al., 2012; Dalton et al., 2009) and

in those with behavioural and learning disorders, such as ADHD (Milte et al., 2011). Therefore, one hypothesis of future research should consider the effect of chronic WBB supplementation on the cognition of underperforming children or those with sustained attention deficits, such as ADHD.

Last, although the effects of chronic WBB treatment over a four-week period were assessed here, this duration may still not be long enough to invoke changes to reading behaviour, hence the absence of reading effects. Indeed, the previous research examining chronic PUFAs supplementation on reading ability in children provides support for the use of longer treatment periods to elicit positive effects when employing a chronic design. Significant improvements to reading ability were observed following PUFAs treatments ranging from three to six months (Richardson & Montgomery, 2005; Richardson et al., 2012; Dalton et al., 2009). As this was the first study examining the chronic effects of berry-flavonoids in children, a shorter duration of four weeks was employed to act as a pilot for future research, as well as to maximise recruitment. Although chronic WBB intake improved executive function reaction time over the four-week period, a finding in line with previous chronic berry-flavonoid studies where significant improvements were observed following treatment ranging from five weeks to four months (Bowtell et al., 2017; Nilsson et al., 2017; Miller et al., 2019), this four-week period was not long enough to induce reading effects in this instance. Therefore, future research should consider longer periods of WBB supplementation to keep in line with the PUFAs literature in order to examine the chronic effects of WBB supplementation on reading behaviour.

To conclude, the chronic effects of a daily 30 g freeze-dried WBB powder treatment consumed over a four-week period significantly improved executive function reaction times (ms; correct responses only) for the more cognitively demanding fast trials. Here, faster reaction times were observed after four weeks WBB consumption compared to two weeks consumption, and compared to placebo. Chronic WBB supplementation also exhibited a potential maintenance effect for both positive affect and negative affect which remained constant over the four-week period; placebo significantly reduced positive affect and increased negative affect. Acute WBB intake did not improve cognition, reading behaviour, or mood. Chronic WBB supplementation had no effect on word list recall or reading behaviour. Several methodological issues such as sample size, testing environment, nature of recruitment, were put forward to explain the lack of acute findings, which may further

have influenced the chronic findings. Future replication is needed, paying particular attention to overcoming the methodological issues raised here to examine the full potential of chronic WBB supplementation on cognition, reading behaviour, and mood, in children. Further, future research should consider focusing on a different aspect of reading when examining the effects of berry-flavonoid treatments on reading behaviour. One example put forward by the present study is to examine the act of learning to read words from context which highlights how children learn to read words naturally; a measure similar to the AVLT which assess the list learning of known words.

With this in mind, Study 4 will aim to examine the effects of acute WBB supplementation on the ability of seven to eight-year olds to learn to read words from context. This will allow the examination of an acute berry-flavonoid intervention on the initial stages of word reading: the learning of novel orthography and phonology, instead of the end of product of reading: the fluency and automaticity of reading as assessed by the TOWRE-2.

Chapter 5

Effects of acute wild blueberry supplementation on word learning in eight to nine-year olds.

5.1 Introduction

Thus far, the findings from Studies 1 to 3 have failed to show either acute or chronic WBB improvements to word reading, in particular to the sight word reading of known words or the phoneme decoding of non-words. As discussed previously (Section 4.5), it is possible that WBB supplementation may not benefit word reading, or that the measure used to assess word reading, the TOWRE-2, was not sensitive enough to detect subtle changes to word reading following a flavonoid-rich WBB treatment. However, this does not rule out the possibility that WBB supplementation benefits reading behaviour in some way.

As outlined in Chapter 1, the ability to learn to read words is one of the first steps to becoming an established reader and focuses on the learning of essential mappings between orthography and phonology to produce a correct pronunciation (Ehri, 1995). To date, research has not examined the effect of dietary interventions on learning to read novel words, however acute WBB effects have been seen consistently for the list-wise recall and recognition of known words (Whyte et al., 2016; Studies 1 & 2); improvements and maintenance effects on word list recall performance and executive function were observed up to six hours post WBB consumption. These findings suggest that acute WBB supplementation has the capability to improve some aspects of visual word learning such as the list-wise recall and recognition of known words. Therefore, it is possible that acute WBB intake may be able to improve the process of learning to read novel words.

5.1.1 Learning to read words

The first step in learning to read words involves children gaining an understanding of the alphabetic principle (Ehri, 1995). This principle teaches the connections between letters of the alphabet and their associated phonology, paving the way for the decoding of newly encountered words using a letter-by-letter approach. Indeed, when children encounter a new word in text, the novel word is learnt through decoding repetition until the word and its pronunciation are learnt and stored in the reader's internal lexicon ready for later retrieval. This process of self-directed learning is described by the self-teaching hypothesis (Share,

1995), an influential theory of orthographic learning detailing how children learn to read words. Share (1999) developed an orthographic learning paradigm to assess the self-teaching hypothesis, a paradigm which has been widely used since. In general, children learn novel words within a story context, mimicking the natural process by which children learn new words whilst reading. Orthographic learning is then assessed by orthographic post-tests where children are asked to name (read), spell, or identify the novel word spellings in an orthographic choice task.

Recent research utilising this orthographic learning paradigm set out to explore individual differences in children's orthographic learning. For example, Ricketts et al. (2011) assessed the orthographic learning of seven to eight-year olds when non-words were repeatedly presented in individual story contexts. Orthographic learning was assessed by an orthographic choice task and a spelling task. Standardised measures of reading and existing vocabulary knowledge were further completed to examine potential predictors of orthographic learning. Ricketts et al. (2011) observed a significant increase in orthographic learning over a number of exposure trials, suggesting the successful learning of orthography-phonology mappings for the non-words; in line with the self-teaching hypothesis (Share, 1995). Further, significant positive correlations between orthographic learning and decoding, reading comprehension, and text reading accuracy, were observed. This suggests a greater ability to learn the orthography-phonology mapping of non-words is positively associated with greater reading ability in general.

Further, work from Mimeau et al. (2018) supports this association between orthographic learning, word reading, and reading comprehension. Children aged eight to nine years old learnt non-words when presented in individual story contexts in an adapted orthographic learning paradigm (Bowey & Miller, 2007; Ricketts et al., 2011; Wang et al., 2011). Additional word reading fluency, accuracy, and reading comprehension measures were assessed prior to the orthographic learning paradigm. After an initial exposure phase, performance on later orthographic learning post-tests significantly correlated with word reading performance, indicating a direct effect of orthographic learning on word reading. An indirect effect of orthographic learning was further observed on reading comprehension via word reading, again highlighting the importance of efficient word learning for established word reading and reading comprehension.

Wang et al. (2011) set out to examine whether the story context in which novel words are presented could aid learning to read words. Essentially, does contextual support offered by the orthographic learning paradigm contribute to orthographic learning? Specifically, this study focused on whether there was greater facilitation of word, non-word and irregular non-word learning in context compared to list format. Here, seven to eight-year olds demonstrated significantly greater reading accuracy when non-words were presented in context compared to isolation, as well as showing a moderate benefit of context on orthographic learning for irregular non-words. No effect of context was found for regular novel words. This suggests that the surrounding context plays a role in facilitating the orthography-phonology mappings of non-words with and without irregular spelling patterns where prior vocabulary knowledge may not prove beneficial. This supports Ehri's (1995) notion that sophisticated strategies of learning to read words, such as the use of contextual support, are adopted by readers dependent on their experience with print and reading in general.

Orthographic learning research not only assesses immediate performance, but also assesses the retention of orthographic knowledge over time. For example, Wang et al. (2011) assessed orthographic learning immediately after exposure to non-words and ten days later to assess the delayed retention of the learnt non-words. Both spelling and orthographic choice showed greater accuracy for correct responses immediately after non-word exposure compared to delayed retention. Indeed, a higher number of recognition errors were made during the delayed test session whereby a greater number of alternative foils were chosen compared to the target stimuli, and compared to immediate testing. Wang et al.'s (2011) findings suggest a disruption to orthographic learning retention ten days after exposure compared to immediate testing. Similarly, Mimeau et al. (2018) also assessed delayed retention approximately one to nine days following immediate testing after non-word exposure. It was observed that the longer the delay between the two test sessions, the lower the scores were for both word reading and reading comprehension at the delayed test session. This suggests that the longer the period between exposure and retention, the poorer accuracy performance will be during the delayed test session, subsequently leading to poorer word learning ability.

5.1.2 Berry flavonoids and visual word learning

To date, no effects of acute WBB treatment on word reading have been observed (Studies 1-3). However, acute WBB supplementation has been shown to significantly improve the list-wise recall and recognition of known words, as assessed by the AVLT, one to two hours after treatment (Whyte & Williams, 2015; Whyte et al, 2016; Studies 1 & 2). The AVLT involves hearing lists of regular words before recalling these words orally and recognising them visually, thus assessing the encoding, retention, and retrieval, of regular orthography. Similar to the AVLT, orthographic learning paradigms are often used to assess the encoding and retrieval of novel orthography. As stated above, orthographic learning paradigms require the visual learning of new words before recognising them visually through an orthographic choice task or recalling their spelling after auditory presentation. Here, the essential orthography-phonology mappings of novel words are learnt in story context to mimic how children learn new words when reading. The similarities of these measures suggest that, as acute WBB intake has the potential to improve the encoding and retrieval of regular words, it may further have the potential to improve the encoding and retrieval of novel non-words. Further, acute WBB supplementation has been seen to improve the list-wise learning of regular words as assessed by the AVLT. This further suggests that acute WBB intake may have the potential to improve the learning of novel words when presented in story context. Indeed, Wang et al. (2011) provides support for this as observed significantly greater facilitation of non-words when presented in context compared to words, and when presented in a list format.

5.1.3 The present study

An acute randomised, double-blind, between-subjects, placebo-controlled design was implemented to examine the effect of an acute 13 g freeze-dried WBB powder treatment (253 mg anthocyanins; Section 5.2.3) on visual word learning in eight to nine-year old children. Participants of this age were recruited in line with prior work examining orthographic learning in children (Bowey & Miller, 2007; Cain et al., 2003, 2004; Ricketts et al., 2008; 2011). At this age, orthographic skills become established (Juel et al, 1986) and most children have sufficient reading skills to read to acquire new information (Chall, 1983). Children understand phonemic awareness and grapheme-phoneme connections, deeming them to be fully functioning at the full and consolidated alphabetic phases of reading

development (Ehri, 2005). Here, they are not only able to learn new words when presented orally (when heard), but also visually (when read).

To assess word learning, the present study employed an orthographic learning task to simulate how children learn new words whilst reading. Visual word learning performance was assessed two hours and 24 hours after treatment consumption using an orthographic choice task and a spelling task. Taking the above literature into account, it was first hypothesised that acute WBB supplementation would positively benefit visual word learning when assessed by the orthographic learning post-tests. Second, when considering the changes to orthographic learning observed previously between an immediate test session and delayed retention testing, it was further hypothesised that there would be a difference in visual word learning performance between the two-hour and 24-hour test sessions (Ricketts et al., 2011; Wang et al., 2011; Mimeau et al., 2018).

5.2 Methods

5.2.1 Participants

For the present study (for ethical approval see Appendix A.2), an *a priori* power analysis (using G Power 3.1) based on the significant AVLT total word recall finding from Study 1 was used to calculate the required sample size. A two-tailed test with a partial alpha of $p = .05$ revealed a total of 42 children would be required to achieve sufficient power of 0.8 ($n = 21$ per treatment group) when employing a between-subjects design.

A total of 52 healthy children (26 males: 26 females) aged eight to nine years ($M = 8.26$, $SD = .40$) were recruited from three primary schools local to the University of Reading. Written consent was obtained from parents/legal guardians (see Appendices B.3 & C.3) prior to participation, as well as participant's assent (see Appendices D.3 & E). Parents/legal guardians confirmed that their child spoke English as a first language, had no known learning or behavioural difficulties (e.g. ADHD, dyslexia or reading impairments), and had no fruit or fruit juice intolerance.

5.2.2 Background measures

Participants completed a series of background measures during an initial practise session 24 hours prior to the first test day: the matrices component of the Wechsler Abbreviated Scale of Intelligence 2nd edition (WASI-II) as a measure of non-verbal intelligence, the vocabulary component of the WASI-II as a measure of breadth and depth of existing spoken

vocabulary knowledge, both TOWRE-2 SWE and TOWRE-2 PDE (see Appendix J) as measures of sight word reading efficiency and phonemic decoding efficiency. Last, participants read aloud one passage from the York Assessment of Reading for Comprehension (YARC; see Appendix G) as a general measure of reading accuracy, reading rate, and reading comprehension.

Twelve participants were excluded after the practice session as performance on the WASI-II and TOWRE-2 showed they were performing below the average range for their age group. These participants were excluded as low scores on these measures indicated potential difficulties in completing the orthographic learning task. Raw scores and standard scores of the background measures for the remaining 40 participants (18 males: 22 females) are shown in Table 5.4.1.

5.2.3 Treatments

For the present study, a new batch of freeze-dried WBB powder was used to make the WBB treatment. This batch contained different concentrations of anthocyanins to the batch used previously in Studies 1 to 3. In order for the present WBB treatment to contain the same amount of anthocyanins as seen in the previous 30 g freeze-dried WBB powder treatment, where positive benefits to cognition have been observed, a reduced overall weight of powder was used (Table 5.2.3). As a result of the reduced overall weight, the WBB treatment had subsequently smaller concentrations of fructose, glucose and vitamin C. No differences in palatability were noticed between the two WBB treatments used in this thesis and no participants abstained from consuming either WBB treatment.

Table 5.2.3: The differences in treatment constituents between the old batch and new batch of WBB used.

	Old Batch		New Batch	
	Placebo	WBB	Placebo	WBB
Total WBB powder (g)	---	30	---	13
Total anthocyanins (mg)	---	253	---	253
Vitamin C (ml)	4	4	.045	.045
Fructose (g)	8.9	8.9	4.78	4.78
Glucose (g)	7.99	7.99	4.52	4.52
Water (ml)	200	200	200	200
Low-flavonoid orange squash (ml)	30	30	30	30

An acute double-blind, randomised, placebo-controlled, between-subjects design was applied with participants randomly allocated to receive either a 13 g freeze-dried WBB powder treatment or a sugar and vitamin-C matched placebo (*n* = 21 WBB, *n* = 19 placebo). The 13 g freeze-dried WBB powder treatment (equivalent to 240 g fresh WBB) contained 253 mg anthocyanins. The placebo contained fructose (4.78 g), glucose (4.52 g) and vitamin C (45 mg) to match concentrations found in the 13 g WBB treatment. See Section 2.2.3 for further information on treatment preparation and administration.

5.2.4 Word learning procedure and measures

5.2.4.1 Stimuli

Participants were exposed to eight four-letter monosyllabic non-words embedded in written story contexts which described a fictional professor's new inventions. Non-words and stories were taken from previous studies (Ricketts et al., 2011; Mimeau et al., 2019; Wang et al., 2011). Each non-word was presented in its own story and was repeated four times within the story (see Appendix O). For example*, "*Ben was at the pet shop and the fish

tank looked dirty. Ben picked up the [non-word]. The [non-word] is used to clean fish tanks. Ben placed the [non-word] into the fish tank. When the tank was clean, Ben put away the [non-word]."

The stories provided participants with opportunities to learn associations between the non-words and their novel meanings. Non-words were used to minimise the effect of prior knowledge on learning. Each non-word named one of 'Professor Parsnip's' inventions (e.g., nawn). Non-words were selected that had two homophonic spellings (e.g. nawn and norn) to generate a target non-word and an alternative foil for the orthographic choice post-test (Section 5.2.4.3.1). This provided two sets of non-word stimuli (Table 5.2.4.1) which were counterbalanced between participants; half the participants learnt Set A whilst the remainder learnt Set B.

Table 5.2.4.1: Non-word stimuli used for the orthographic learning task.

	Non-words							
Set A	nawn	lort	goap	hode	jeel	meab	ferg	surk
Set B	norn	lawt	gope	hoad	jeal	meeb	furg	serk

5.2.4.2 Exposure phase

Prior to learning the non-words on day one, a practise story (see Appendix O) was presented. For this story, children were told they would be reading about something they already had knowledge of (e.g., sheep); they were provided with positive feedback after reading this story.

After the practise story participants were told they would be learning about 'Professor Parsnip's' new inventions. They read aloud the eight stories with stories presented one at a time on a computer screen and with the order of presentation randomised amongst participants. Feedback was not given for test stories. Non-word exposure did not occur on day two.

5.2.4.3 Orthographic learning post-tests

5.2.4.3.1 Orthographic choice task

Participants were first presented with a 1 second fixation slide before a non-word pair was presented. The target (e.g., nawn) and homophonic alternative foil (e.g., norn) for each non-word were presented for an unlimited time on a computer screen and appeared in coloured boxes to the left (green) and to the right (blue) of a central fixation cross. Participants were instructed to press the corresponding colour key on a computer keyboard to correctly identify the target non-word learnt previously in the exposure phase. The position and order of the non-word targets and alternative foils were randomised between test sessions and participants. The outcome measures for the orthographic choice task were choice accuracy (the proportion of correct responses with a score of 1 equivalent to 100% correct responses) and reaction time for correct responses only. Reaction times for correct responses only included responses if above 100 ms and trimmed such that reaction times >2 SDs outside of each child's mean were discarded. Reaction times >2 SDs were likely to be spurious responses not made in relation to the stimuli but rather by error or reflex. Therefore, inclusion of these reaction times would have distorted the data and given a false impression of the effect of WBB on performance.

5.2.4.3.2 Spelling task

Participants were instructed to spell the previously learnt non-word stimuli to dictation on paper provided. Participants were first asked to spell four practise words to dictation (e.g. sheep, rock, house, bird). This was to ensure that participants understood what was expected of them before being ask to spell the non-words. The non-words were presented in a randomised order between test sessions and participants. The outcome measure for the spelling task was accuracy of spelling of the whole word. The overall number of words spelled correctly was used to calculate a participant's total spelling accuracy score.

5.2.4.4 Procedure

5.2.4.4.1 Background session

Participants took part in a screening session 24 hours prior to treatment consumption and testing on day one. Here, participants completed the background measures mentioned previously: non-verbal intelligence, SWE and PDE, vocabulary, and general reading ability.

After completion of the background measures, participants were asked to follow a 48-hour low-flavonoid diet (see Appendix F) before completing the remainder of the study. An information sheet detailing high-flavonoid foods to avoid and a list of low-flavonoid alternatives was given to parents/legal guardians to maximise compliance. Parents were contacted to remind them of compliance with the 24-hour low-flavonoid diet. No baseline test session was conducted in order to reduce the effect of practise on orthographic learning performance, in line with Mimeau et al. (2018).

5.2.4.4.2 Day one

Participants consumed their randomised treatment at either 0900 hours or 0930 hours, before returning to class for a two-hour period. During this period, consumption of any food or beverage (other than water) was not permitted and all participants were asked to abstain from exercise. Two hours after consumption of their treatment, participants completed the first test session at either 1110 hours or 1140 hours (dependent on time of treatment consumption) which lasted approximately 30 minutes. Participants read the eight stories that contained the non-word stimuli and completed the orthographic choice and spelling post-tests immediately afterwards. Testing took place individually in a quiet room with all tasks computerised, apart from spelling responses. After completing the first test session, participants were reminded to comply with the low-flavonoid food diet for the remainder of the study (24 hours).

5.2.4.4.3 Day two

Participants were invited back 24 hours after treatment consumption. For this second test session participants were not exposed to the non-word stimuli in story context but immediately completed matched versions of the orthographic choice and spelling post-tests to assess the retention and retrieval of the learnt non-words over the 24-hour period. This session took place at either 1110 hours or 1140 hours (dependent on time of treatment consumption on day one) and lasted approximately 15 minutes. Once finished, participants were thanked for their participation and both child and parents were fully debriefed via debrief information sheets sent home by the school.

5.3 Statistical analysis

Data were analysed using SPSS (Version 21.0). Differences between treatments (WBB, placebo) for background variables: WASI-II matrices component, WASI-II vocabulary component, TOWRE-2 SWE and PDE, and YARC, were examined using independent-samples t-tests. A chi-square was performed to assess sex differences between the treatment groups. Mean, standard deviations and group comparisons are presented in Table 5.4.1.

Linear Mixed-effects Models using unstructured covariance matrices to model repeat test sessions were used to analyse data for three dependent variables: orthographic choice accuracy, orthographic choice reaction time (correct responses only), and spelling accuracy. A separate model was used to analyse each dependent variable.

For the present study, LMM was used to address the hypotheses for interest. For all models, Treatment was included as a fixed factor to explore the difference between WBB and placebo. Session was also included in all models as a fixed factor to explore the difference between the immediate retrieval and delayed retrieval of the learnt non-words. Interactions between Treatment and Session were examined for all three outcome measures to assess whether performance differed between sessions for each treatment or between treatments for each session. The random effects of participants on intercepts was included in all models to control for individual variation around the by-participant mean. The random effects of items on intercepts was also included in all models to control for the individual variation of each non-word stimuli around the by-item mean. In all models, pairwise comparisons were used where appropriate and Bonferroni corrections were applied to control for type I error.

5.4 Results

5.4.1 Background measures

The below table (Table 5.4.1) describes the means, standard deviations, and ranges of raw scores and standard scores for background measures assessed prior to testing, as well as key demographic information such as age and gender.

A significant difference was also observed between groups for YARC reading rate raw scores; $t(38) = 3.41$, $p < .005$, $d = .78$, where those receiving WBB had significantly slower reading rates than those receiving placebo. A significant difference was further observed

between groups of YARC reading comprehension raw scores; t(38) = -2.66, p = .011, d = .86. In contrast, those receiving WBB had significantly poorer reading comprehension scores than those receiving placebo. These findings suggest that participants consuming WBB may be substituting greater comprehension for a faster reading rate, and vice versa for participants consuming placebo. YARC standard scores were used to characterise the sample only, raw scores were used to analyse differences between the treatment groups. In order to calculate YARC standard scores, participants are required to read two YARC passages, where the raw scores are then combined to create an average ability score. This average ability score is then converted using conversion tables provided by the YARC manual (Snowling et al., 2009) into a standard score. As participants only read one passage (in order to reduce the duration of the practise sessions), a rough standard score was calculated for each participant in order to characterise the sample and to compare this cohort of children to those recruited in Studies 1 to 3.

No significant differences between treatments were observed for: YARC accuracy, TOWRE SWE and PDE, WASI-II matrices or vocabulary. Overall, the groups were well matched for age gender, TOWRE, WASI-II matrices and vocabulary but not for the YARC measures.

When considering performance on the WASI-II, this cohort achieved average t scores for both the matrices (WBB: M = 47.76, placebo: M = 49.58) and vocabulary (WBB: M = 47.95, placebo: M = 49.9) components. These findings suggest that children were performing within the norm (M = 50, SD = 10) for non-verbal intelligence and for the breadth and depth of existing spoken vocabulary knowledge. Age normed scores from the TOWRE-2 suggest that this sample of children performed above the average for their age range for SWE (Grades 1-5: M = 104.7, SD = 13.3), whilst performing in line with their age range average for PDE (Grades 1-5: M = 105.9, SD = 13.3). However, the above findings for both the WASI-II and TOWRE-II were expected as the study excluded participants who performed below average for their age range. Further, both treatment groups performed in line the age norm for all YARC components, with placebo also performing one standard deviation above the norm for their age group for reading accuracy. This indicated that all children had average to good reading ability and were matched to the children in Studies 1 to 3 for reading accuracy, reading rate, and reading comprehension.

Table 5.4.1: Data for background measures and demographics including raw score means, standard score means, *t* score means, standard deviations, range, *t* statistics, and *p* values.

	Placebo (*n* = 19)			Blueberry (*n* = 21)				
	M	***SD***	**Range**	***M***	***SD***	**Range**	***t***	***p* values**
Age	8.22	.36	8.01-9.10	8.29	.44	8-9.10	.518	.607
Gender (male:female)	9:10	--	--	9:12	--	--	---	--
Wechsler Abbreviated Scale of Intelligence Matrices								
Raw scores	14.26	3.69	9-22	13.9	3.13	9-19	---	---
t scores	49.58	8.6	38-70	47.76	5.99	38-59	-.781	.440
Vocabulary								
Raw scores	21.37	5.78	8-28	22.67	4.48	14-32	---	---
t scores	47.95	9.67	27-60	49.9	7.56	37-64	.717	.478
Test of Word Reading Efficiency Sight word efficiency (SWE)								
Raw scores	107.95	9.59	90-128	108.43	11.38	89-131	.14	.886

Table 5.4.1: *continued…*

	M	*SD*	Range	*M*	*SD*	Range	*t*	*p value*
Phonemic decoding efficiency (PDE)								
Raw scores	104.53	14.82	84-128	105.86	12.81	85-130	.31	.762
York Assessment of Reading for Comprehension								
Reading Accuracy								
Raw scores	5.53	4.82	0-21	6.38	6.02	0-19	.492	.626
Standard scores	110.11	11.53	85-126	109.29	12.61	84-130	-.214	.831
Reading Rate								
Raw scores (ms)	94.42	23.99	66-139	148.67	65.38	71-321	3.41	.002*
Standard scores	118.11	11.99	91-130	106.81	14.35	89-130	-2.69	.011*
Reading Comprehension								
Raw scores	7.00	0.88	5-8	6.09	1.22	3-8	-2.66	.011*
Standard scores	101.05	3.34	96-107	98.76	4.37	91-108	-1.87	.072

Notes. WASI-II t scores (M = 50, SD = 10). YARC standard scores (M = 100, SD = 15). No significant differences were observed between the number of males and females receiving each treatment ($\chi 2 = .018$, $p = .548$)

5.4.2 Orthographic choice task accuracy

When collapsed across treatment groups, the collective sample were shown to have greater accuracy performance at two hours compared to 24 hours. Means and standard deviations are presented in Table 5.4.2A.

Table 5.4.2A: Means and standard deviations for orthographic accuracy (proportion of correct responses where 1 is equivalent to 100% correct responses) at two hours and 24 hours post-treatment.

	Placebo (n = 19)		**WBB (n = 21)**	
	M	***SD***	***M***	***SD***
Two hours	.72	.45	.72	.45
Twenty-four hours	.63	.49	.62	.49

When assessed independently, the fixed effect of Session was a significant predictor of orthographic choice accuracy; F(1,320.02) = 6.36, MSE = .05, p = .012, n^2 = .14, with pairwise comparisons revealing significantly greater accuracy for detecting target stimuli at two hours compared to 24 hours, regardless of treatment (Table 5.4.2B).

The fixed effect of Treatment did not significantly predict orthographic accuracy, and there was no significant Treatment x Session interaction.

Table 5.4.2B: β coefficients and p values for orthographic choice task accuracy (%) LMM analysis.

Fixed effects	**β coefficient**	***p* value**
Treatment	-.005	.957
Session	.088	.012
Treatment x Session	.005	.945

5.4.3 Orthographic choice task reaction time (ms: correct responses only)

On average, it took participants 2.5 to 3 seconds to decide between the orthographic target and foil in this task. When collapsed across treatment groups, the collective sample were shown to have faster reaction times to correct responses at 24 hours compared to testing at two hours. Means and standard deviations are presented in Table 5.4.3A.

Table 5.4.3A: Means and standard deviations for orthographic reaction times (ms; correct responses only) at two hours and 24 hours post-treatment.

	Placebo (n = 19)		**WBB (n = 21)**	
	M	***SD***	***M***	***SD***
Two hours	2394.77	995.99	2924.69	1159.66
Twenty-four hours	2050.81	751.01	2302.61	1001.39

The fixed effect of Session significantly predicted orthographic choice task reaction times when assessed independently; F(231.59) = 38.47, MSE = 118.66, $p < .001$, $n^2 = .76$ (Table 5.4.3B). Here, pairwise comparisons revealed that participants were significantly faster at identifying the target stimuli at 24 hours compared to two hours.

The Treatment x Session interaction showed a near significant trend; F(1,231.60) = 2.84, MSE = 159.50, $p = .094$, $n^2 = .07$. Pairwise comparisons revealed significantly faster reaction times at 24 hours compared to two hours for both placebo ($p < .005$) and WBB ($p < .001$).

The fixed effect of Treatment did not predict orthographic learning reaction times.

Table 5.4.3B: β coefficients and *p* values for orthographic choice task reaction times (ms; correct responses only) LMM analysis.

Fixed effects	**β coefficient**	***p* value**
Treatment	1456.39	.283
Session	360.37	< .001
Treatment x Session	268.58	.094

5.4.4 Spelling

Overall performance for spelling accuracy (the number of whole words spelled correctly) initially indicated no observed difference between treatments or sessions suggesting that spelling accuracy remained stable over the 24-hour period. Means and standard deviations are presented in Table 5.4.5A.

Table 5.4.5A: Means and standard deviations for spelling accuracy (the number of whole words spelled correctly) at two hours and 24 hours post-treatment.

	Placebo (n = 19)		**WBB (n = 21)**	
	M	***SD***	***M***	***SD***
Two hours	.44	.50	.46	.49
Twenty-four hours	.49	.50	.46	.50

The fixed effects of Treatment and Session were not significant predictors of spelling accuracy, and there were no significant interactions between the fixed effects. No pairwise comparisons were conducted for this non-significant interaction (Table 5.4.4B).

Table 5.4.4B: β coefficients and *p* values for spelling accuracy LMM analysis.

Fixed effects	**β coefficient**	***p* value**
Treatment	-.03	.921
Session	.04	.440
Treatment x Session	.04	.440

5.5 Discussion

The present study aimed to examine the effect of acute WBB supplementation on learning to read words in eight to nine-year old children when presented in story context. The study hypothesised that acute WBB supplementation would impact the learning of novel

orthography, and that there would be a difference in word learning performance between two hours and 24 hours. No effects of acute WBB supplementation were observed for learning to read words at two hours or 24 hours. Non-word retrieval did differ significantly between the two test sessions, regardless of treatment. In general, greater orthographic choice accuracy was observed at two hours whilst faster orthographic reaction times were observed at 24 hours. Spelling performance did not differ between sessions, regardless of treatment.

The null effect of acute WBB supplementation on learning to read words is consistent with the previous research on word reading within this thesis (Studies 1-3), thus indicating that acute WBB interventions do not benefit either word reading or learning to read words in children. Although acute benefits to word list recall and recognition have been previously observed in the child literature following acute WBB intake (Whyte and Williams, 2015; Whyte et al., 2016; Studies 1-3), the present study demonstrates that learning to read novel visual words in story context, and thus mimicking the natural process by which children learn to read, was not affected by acute WBB consumption. To conclude this line of research, future work could examine the effect of chronic WBB supplementation on learning to read words. Although previous research failed to show chronic effects of WBB supplementation on word reading as assessed by the TOWRE-2 (Study 3), effects of chronic WBB supplementation on learning to read words might be plausible when the methodological issues noted in Study 3 are addressed. For example, an adequate sample size is needed in order to produce a study with appropriate power and effect. Indeed, the potential for chronic WBB consumption to improve learning to read words is supported by the previous literature where chronic PUFAs supplementation significant improved reading behaviour in children (Richardson et al., 2012; Dalton et al., 2009; Johnson et al., 2017). This was observed for measures assessing children's previous knowledge of letter-sound correspondences and potential sight word reading of already known words (Richardson et al., 2012; Word Reading Achievement sub-test of the BAS), orthographic recognition (Dalton et al., 2009; Hopkins Verbal Learning Task) and phonemic decoding time and visual analysis time (Johnson et al., 2017; The Logos Test).

In relation to the test sessions, Session was a significant predictor of both orthographic choice accuracy and reaction time for correct responses. Here, accuracy was greater at two hours in comparison to 24 hours, whilst reaction time for correct responses was slower at

two hours in comparison to 24 hours. One potential reason for the differences observed between the two test points may be the novelty of the stimuli. At two hours participants learnt a set of target non-words before being presented with an alternative set of homophonic foils during the orthographic choice task. The presentation of the alternative set at two hours immediately after learning the target non-words may impede and interfere with their learning and encoding. This principle can also be applied to any additional learning within the classroom after the two-hour test session which may further interfere with the learning of the stimuli. This rationale is supported by anti-priming effects (Marsolek, 2008) where learning new stimuli interferes with the encoding and retrieval of previously learnt stimuli, and by superimposing (Rass & Leynes, 2007) where words with similar visual representations (such as would be shared by the two sets of homophonic non-words) impede each other's learning. Reduced orthographic choice accuracy at 24 hours could be the result of forgetting. This coincides with the findings from Wang et al. (2011) and Mimeau et al. (2018) where accuracy performance on the orthographic learning tests declined between immediate testing and delayed retention testing. A similar pattern was observed from the AVLT raw data collected from recalls A1-A7 in the previous chapters (Studies 1-3). Throughout recalls A1-A5, participant word recall increases, however after the distractor list is presented (list B), word recall performance at recalls A6-A7 is reduced due to retroactive interference created by list B.

In comparison, participants exhibited faster reaction times at 24 hours relative to two hours. Here, children were now familiarised to both sets of non-words following repeated presentation in the orthographic post-tests at two hours. This finding was also supported by the near significant Treatment x Session interaction observed for orthographic learning reaction times (correct responses only), with pairwise comparisons revealing significantly faster reaction times at 24 hours compared to two hours for both treatment groups. Therefore, initial interference caused by the alternative set at two hours was reduced, leading to faster reaction times at 24 hours. This increase in reaction time to the target stimuli could be a result of practise effects whereby participants now fully understand the nature of the task and what to expect. However, this effect of practise did not extend to orthographic choice accuracy. This suggests that less time was spent on distinguishing between the target and foil, increasing the likelihood of making an identification error. Indeed, this is supported by the reduced accuracy observed at 24 hours of which forgetting

and retroactive interference are thought to play a role. One further explanation for this pattern of faster reaction times but poorer accuracy at the 24-hour test point could have been the result of boredom/fatigue effects, leading to impulsivity and guessing, rather than an effect of treatment on performance.

An initial baseline difference was observed between the treatment groups for the YARC components. Those receiving WBB showed significantly greater reading comprehension compared to placebo. However, significantly slower reading rates were also observed in the WBB group compared to placebo. Together, these findings suggest that the WBB group compromised a faster reading rate for greater reading comprehension due to being more attentive in their performance, whilst the placebo group showed the reverse. Another explanation for the significantly worse reading comprehension scores following acute WBB intake is that the WBB group found the YARC passage reading slightly harder than the placebo group. If attention was solely focused on reading the passage correctly then this could account for the WBB group having poorer reading comprehension. Here, attention would have been taken away from understanding the passage as a whole to focus on correct reading. Nonetheless, both treatment groups showed no significant difference in baseline performance on the other measures of reading behaviour, such as YARC accuracy, the TOWRE-2 and WASI-II vocabulary subtest. This suggests that the reading of the passage itself, and how difficult participants found it, may have been the result of these group differences. Further, it is important to note that raw scores were used to analyse these results, meaning that findings are not standardised to age norms or comparable to other research (Section 5.4.1). In order to accomplish this, standard scores would need to be computed, therefore the differences in raw scores should be interpreted with caution. Last, although the difference in YARC reading comprehension between the treatment groups was significant, the difference between the means (WBB: M = 6.09, placebo; M = 7) and the reported effect size (d = .21) being small. This suggests that the significant difference in reading comprehension may not be meaningful and should be interpreted with caution. Although not measured in this study, it would be interesting to examine whether similar differences were apparent in children's ability to read aloud the stories used by the orthographic learning task. Overall, it is unlikely that these group differences between YARC components influenced the results, as both treatment groups were still performing within or above the age range.

One limitation thought to explain the nature of the results obtained here is the sample recruited. At present, only participants performing in line with or above the average performance age range for TOWRE-2 and WASI-II were included. Previous research assessing orthographic learning states that prior orthographic knowledge contributes to orthographic learning (Pacton et al., 2001), such that greater prior knowledge equals greater learning. To this effect, Ricketts et al. (2011) demonstrated that TOWRE-2 performance was a trending predictor of orthographic choice task and spelling performance further suggesting that the sample included here would have greater performance on the orthographic learning tasks than others with poorer performance. As no effects of acute WBB supplementation were observed in the present study, it would be prudent to examine whether acute WBB intake can benefit the orthographic learning of those with below age average reading ability. Indeed, it is in such a sample that previous dietary intervention studies have shown a benefit to word reading (Richardson et al., 2010; Zhang et al., 2005).

Further benefits of acute WBB consumption for learning to read words may also be observed for younger children. The children recruited for the present study were fully functioning in the full or consolidated alphabetic phases of Ehri's (1995) phase development, whereby they have a concrete understanding of GPC rules and thus are sufficient at decoding. In line with the theory above that acute WBB consumption may benefit children with below average reading ability, acute WBB intake may be able to benefit those who are just starting to learn to decode. The previous cognitive findings (Whyte et al., 2017; Chapters 2-4) observed a significant improved to executive function reaction time following both acute and chronic WBB supplementation. Therefore, acute WBB consumption may have the potential to improve the reaction times of decoding attempts when a child is learning to decode, such as when children are in the partial alphabetic phase. Here, GPC rules are not completely consolidated in the reader's mind, although they generally have the ability to decode and recognise first and last letters within a word. Alongside the usual methods of phonics instruction (systematic phonics instruction and phonological awareness), acute WBB supplementation may have the potential to increase the rate at which decoding of the whole word takes place, thus speeding up the recognition of letter-sound correspondences.

To conclude, acute WBB supplementation in eight to nine-year olds did not significantly impact learning to read words when presented in a story context. This is the first study to examine such an interaction between berry-flavonoids and word learning, or the use of a

dietary intervention on word learning in general. The cognitive domains underlying word reading and visual word learning are known to be susceptible to berry-flavonoid interventions so highlight the possibility for said interventions to benefit visual word learning. In addition, cognitive tasks applying similar cognitive strategies to visual word learning, such as the AVLT, specifically demonstrate benefits to word learning through list-wise recall and recognition. However, as of yet the limited amount of research into this area, such as that conducted in this thesis, has failed to support this hypothesis. The research conducted thus far has only focused on typical populations whose performance is within or above the age average, therefore the next stage of this research should consider whether berry-flavonoid interventions can aid children with poor reading performance; this is consistent with previous dietary intervention studies on word reading where benefits have been observed for those underperforming at baseline (Richardson et al., 2012). In general, the findings from the child literature exploring the effects of WBB supplementation on cognition is novel in itself so as the field progresses, we may gain a clearer understanding of how WBB supplementation is positively benefitting the cognitive processes underlying reading behaviour, and whether this can indeed benefit word reading and learning to read words in the future.

Chapter 6

Final Discussion

Overall, the thesis aimed to examine the effects of acute and chronic WBB supplementation on the cognition, reading behaviour, and mood, of seven to ten-year olds. First, Study 1 examined the acute effects of a 30 g freeze-dried WBB powder treatment (253 mg anthocyanins) on the specific cognitive domains previously shown to be sensitive to acute berry-flavonoid interventions in children: episodic memory as measured by word list recall and recognition, and executive function as measured by the MANT (Whyte & Williams, 2015; Whyte et al., 2016; 2017). In addition, the novel effects of acute WBB supplementation on mood and reading behaviour were further examined throughout this thesis (Studies 1-3). Second, in response to the findings from Study 1, Study 2 considered the effects of time of day, examining a potential interaction between time of day (morning versus afternoon testing) and the acute effects of the 30 g freeze-dried WBB treatment on children's cognitive performance, reading behaviour, and mood. Next, Study 3 examined the chronic effects of the daily 30 g freeze-dried WBB powder treatment consumed over a four-week period on cognition, reading behaviour, and mood. Last, as a result of the null effects of acute and chronic WBB consumption on reading behaviour (Studies 1-3), Study 4 examined the acute effects of a 13 g freeze-dried WBB treatment (253 mg anthocyanins) on children's ability to learn to read new words.

Briefly, this programme of research demonstrated acute WBB-related benefits to episodic memory: total word recall and delayed word recall as measured by list-wise word learning (Studies 1 & 2). Benefits to executive function reaction time were observed following both acute and chronic WBB intake on the more cognitively demanding trials (Studies 1 & 3). Further, time of day was found to interact with acute WBB supplementation, improving executive function reaction times in the afternoon but not in the morning (Study 2). Self-reported positive affect was also seen to improve following acute WBB intake (Study 1), whilst chronic WBB supplementation maintained both positive affect and negative affect over the four-week period (Study 3). However, in this instance, neither acute nor chronic WBB intake affected reading behaviour for word reading (Studies 1-3) or learning to read novel words (Study 4).

6.1 Summary of results

A summary of the main findings from Studies 1-4 is reported in Table 6.1.

6.1.1 Study 1 – Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds

A single-blind, randomised, between-subjects design examined the effects of a 30 g freeze-dried WBB powder treatment (253 mg anthocyanins) on children's performance, compared to a sugar and vitamin-C matched placebo. Performance was assessed at baseline and two hours after treatment consumption. For verbal episodic memory, total word recall and short delayed recall as measured by list-wise word learning significantly improved two hours after WBB intake, compared to placebo. Both measures showed a greater number of words recalled two hours after WBB consumption, suggesting that acute WBB intake aids the immediate encoding and retrieval of words, as well as their delayed retrieval. Acute WBB did not affect performance on any other AVLT outcome measures. Further, a significant Treatment x Target Time interaction for executive function reaction time was observed following acute WBB intake. Although paired comparisons were not significant, this finding indicated that acute WBB intake has the potential to positively benefit reaction time performance by sustaining attention to trials requiring high cognitive demand. Acute WBB did not affect executive function accuracy. Last, a significant increase in self-reported positive affect was observed two hours after WBB consumption, compared to placebo. This finding suggests that acute WBB consumption can boost positive mood in children over a short period of time. No effect was seen for negative affect. No effects of acute WBB supplementation were observed for reading behaviour.

6.1.2 Study 2 - Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds – an interaction with time of day

Study 1 observed similar improvements to Whyte et al.'s (2016) findings for episodic memory and executive function two hours after consumption of the same 30 g freeze-dried WBB treatment. However, subtle differences between the two sets of results were apparent, for example, acute WBB significantly affected performance on different AVLT and MANT outcome measures (Section 2.4). One explanation for these differences could be the time of day when participants were tested and when treatment was administered. For example, Study 1 observed improvements to total word recall two hours after acute WBB

consumption when children were tested in the afternoon. However, Whyte et al. (2016) observed improvements to immediate word recall 75 minutes after WBB intake when children were tested in the morning. Whyte et al. (2016) did not observe acute WBB effects on total word recall at the afternoon test sessions (Section 2.4).

Study 2 replicated the acute procedure and methodology used previously (Study 1), but tested children in the morning. The morning (Study 2) and afternoon (Study 1) data sets were then combined to allow for the direct comparison of performance across time of day, and to examine a potential interaction between time of day and acute WBB supplementation on performance. Consistent with Whyte et al. (2017), executive function reaction times were significantly faster on the more cognitively demanding faster trials two hours after WBB intake when children were tested in the afternoon, compared to the morning. This was further in comparison to the less cognitively demanding trials and to placebo. This finding demonstrates that children sustained greater levels of attention again to the more cognitively demanding trials, but also when experiencing cognitive fatigue such as when tested in the afternoon. Although there were no significant interactions for executive accuracy, a significant effect of time of day was observed where participants tested in the morning had greater accuracy compared to those tested in the afternoon, regardless of treatment.

Further to the time of day effects observed for executive function accuracy, a number of other time of day effects were observed for the episodic memory outcome measures. Indeed, greater performance was observed for words learnt in the morning compared to the afternoon, regardless of treatment. In contrast, greater performance for final acquisition, long delayed word recall, and total delayed word recall was observed in the afternoon compared to morning, regardless of treatment. These findings suggest that performance on measures of immediate word recall is greatest in the morning, whilst performance on measures of delayed word retention is greatest in the afternoon. However, final acquisition only observed a trend towards greater word recall two hours later so this finding should be considered with caution. Next, acute WBB was seen to significantly improve total word recall compared to placebo, regardless of time of day. This finding was in line with that found previously (Study 1) suggesting a consistent effect of acute WBB on immediate word recall in general. The study observed a trend for a Treatment x Time of Day interaction for long delayed word recall, however no significant post-hoc analyses were observed.

Next, although there were no significant interactions for either positive affect or negative affect, time of day independently predicted positive affect. Here, greater positive affect was observed in the morning compared to the afternoon, regardless of treatment. This finding is in line with the previous research on morningness/eveningness chronotypes which demonstrates that children are more alert and attentive in the morning compared to the afternoon (Section 3.5). There was no effect of time of day on negative affect.

Overall, Study 2 observed greater executive function accuracy, episodic memory final acquisition, and positive affect, in the morning, regardless of treatment. Acute WBB supplementation improved total word list recall, regardless of time of day. Acute WBB intake and time of day did not affect reading behaviour for either SWE or PDE.

6.1.3 Study 3 - Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten-year olds

An acute-on-chronic, randomised, between-subjects design was employed to examine the effects of the 30 g freeze-dried WBB powder treatment on cognition, reading behaviour, and mood. Acute testing took place at baseline and two hours after an initial treatment to assess the effects of a single dose of WBB on performance. These acute test sessions replicated the procedure and methodology used previously (Studies 1 & 2). Chronic testing took place following daily WBB consumption over a four-week period, with performance assessed at two weeks and four weeks. This allowed the examination of the accumulative effects of WBB supplementation on performance. Time of day was not standardised across participants at baseline or for the acute and chronic test sessions.

The acute arm of this study observed no effects of treatment on either episodic memory, executive function, mood, or reading behaviour. This was an unexpected finding not in line with the previously observed acute WBB effects for episodic memory and executive function in children (Studies 1 & 2; Whyte and Williams, 2015; Whyte et al., 2016; 2017).

Effects from repeated chronic WBB dosing, however, were apparent. Executive function reaction times were significantly faster for the more cognitively demanding faster trials four weeks after WBB consumption, compared to two weeks and to placebo. This suggests that the accumulative effects of chronic WBB consumption significantly increased sustained attention over the four-week period. Further, Treatment significantly interacted with load and Congruency for executive accuracy performance. Here, significantly greater accuracy

was observed at four weeks compared to two weeks for the less cognitively demanding medium load trials following placebo, and for the less cognitively demanding congruent trials following both treatments. No differences were observed between treatments suggesting that this effect was due to practise. Next, self-reported positive affect significantly decreased following placebo consumption between two weeks and four weeks. In contrast, chronic WBB consumption maintained positive affect and negative affect over the four-week period. This suggests that the accumulative effects of prolonged WBB consumption maintains mood state over a four-week period in children. Last, Session significantly predicted AVLT performance for: short delay word recall, total delayed word recall, and total word recall, with greater performance observed at four weeks compared to two weeks, regardless of treatment. This highlights the influence of practise effects over the four-week period as no effects of treatment were observed.

Overall, chronic WBB supplementation significantly improved executive function accuracy on congruent trials and maintained accuracy between high load and medium load trials, compared to placebo, regardless of session. No chronic WBB effects were observed for word list recall and recognition or reading behaviour.

6.1.4 Study 4 - Effects of acute wild blueberry supplementation on word learning in eight to nine-year olds

Thus far, the previous Studies 1 to 3 failed to show improvements to reading behaviour following either acute or chronic WBB supplementation. One potential explanation for the lack of findings on reading behaviour was the reading measure used. The TOWRE-2 which examines the overall end-product of reading may not have been sensitive enough to detect subtle changes to reading behaviour (Section 2.5). Therefore, Study 4 examined the acute effects of a 13 g freeze-dried WBB powder treatment (253 mg anthocyanins) on a more fine-grained measure of reading: the process of learning to read novel words (orthographic learning). An acute, double-blind, randomised, placebo-controlled design was used. Orthographic learning was measured using an orthographic choice task and a spelling task two hours immediately after treatment consumption and 24 hours later to assess both the immediate retrieval and delayed retention of learnt non-words. There was a trend towards faster orthographic reaction times at 24 hours compared to two hours for both treatment groups. In general, greater orthographic choice accuracy was observed at two hours

compared to 24 hours, regardless of treatment group. No effect of acute WBB was observed for spelling accuracy.

Table 6.1: A summary of Studies 1-4 including: sample size, design, treatments, cognitive test battery, main findings.

Study	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
Study 1 (Chapter 2)	29 female, 25 male mean age 8.24 years	30 g freeze-dried WBB powder treatment (253 mg anthocyanins) or a sugar-matched placebo: fructose (8.9 g), glucose (7.99 g) and vitamin-C (4 ml)	An acute single-blind, randomised, between-subjects design. *n* = 29 WBB, *n* = 25 placebo	Tested at baseline and two hours. Episodic memory (AVLT), executive function (MANT), reading behaviour (TOWRE-2), mood (PANAS-C)	AVLT: acute WBB significantly improved total word recall and short delayed word recall two hours after intake compared to placebo MANT: acute WBB showed a trend towards fast reaction times for the more cognitively demanding fast trials two hours after intake compared to placebo PANAS-C: acute WBB significantly improved positive affect two hours after intake compared to placebo TOWRE-2: acute WBB did not affect SWE or PDE
Study 2 (Chapter 3)	30 female, 31 male mean age 8.20	As reported for Study 1	An acute single-blind, randomised, between-subjects design. Participants: *n* = 31 WBB, *n* = 30 placebo, were tested in the morning. Data was combined with that collected from Study 1.	As reported for Study 1	AVLT: greater performance was observed for words learnt in the morning compared to the afternoon, regardless of treatment. Greater performance for final acquisition, long delayed word recall, and total delayed word recall was observed in the afternoon compared to morning, regardless of treatment. Acute WBB significantly improved total word recall at two hours, regardless of time of day MANT: acute WBB significantly improved reaction times for the more cognitively demanding fast trials when consumed in the

Table 6.1: *continued…*

Study	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
					afternoon, compared to the morning, and compared to placebo. Greater accuracy was observed in the morning compared to the afternoon, regardless of treatment PANAS-C: acute WBB did not affect mood or differ between time of day TOWRE-2: acute WBB did not affect SWE or PDE, or differ between time of day
Study 3 (Chapter 4)	18 female, 12 male mean age 8.21	As reported for Study 1	An acute-on-chronic single-blind, randomised, between-subjects design. *n* = 30 WBB, *n* = 30 placebo	Acute: tested at baseline and two hours. Chronic: tested at two weeks and four weeks. Cognitive test battery as reported for Study 1	Acute: no acute WBB effects were observed for: AVLT, MANT, PANAS-C or TOWRE-2 Chronic AVLT: Greater short delayed word recall, total delayed word recall, and total word recall, was observed at four weeks compared to two weeks, regardless of treatment MANT: WBB significantly improved reaction times for the more cognitively demanding fast trials at four weeks compared to two weeks, and compared to placebo. Significantly greater accuracy was observed for the less cognitively demanding medium load trials following placebo, and significantly greater accuracy was also observed

Table 6.1: *continued…*

Study	Participants	Anthocyanin source	Design	Cognitive tests	Key findings
					for the less cognitively demanding congruent trials following both WBB and placebo. PANAS-C: Positive affect was significantly reduced at four weeks compared to two weeks. Negative affect was significantly greater at four weeks compared to two weeks. WBB maintained both positive affect and negative affect over the four-week period. TOWRE-2: WBB did not affect SWE or PDE.
Study 4 (Chapter 5)	22 female, 18 male mean age 8.26	13 g freeze-dried WBB powder treatment (253 mg anthocyanins) or a sugar-matched placebo: fructose (4.78 g), glucose (4.52 g), vitamin-C (.045 ml)	An acute randomised, double-blind, between-subjects, placebo-controlled design. *n* = 20 WBB, *n* = 22 placebo	Tested at two hours and 24 hours. Orthographic choice task and spelling accuracy of learnt words in a story context	Orthographic choice task: a trend towards faster reaction times at 24 hours compared to two hours was observed following WBB and placebo. Greater accuracy was observed at two hours compared to 24 hours, regardless of treatment. Spelling accuracy: no acute WBB effects were observed.

6.2 General discussion

In this section, the reported acute and chronic cognitive, reading behaviour, and mood effects, are compared to the existing literature. Explanations and limitations are further noted that may have contributed to the differences in findings between the present studies and the existing literature. Implications of the present findings are further discussed.

6.2.1 Cognition

6.2.1.1 Episodic memory

The verbal episodic memory effects observed in Studies 1 and 2 are consistent with findings from the previous child literature (Whyte & Williams, 2015; Whyte et al., 2016). First, both Study 1 and Whyte and Williams (2015) observed similar improvements to delayed word recall two hours after intake of acute berry-flavonoid treatments. Whilst a significant improvement to short delay word recall two minutes after consumption of the acute WBB treatment (253 mg anthocyanins) was observed in Study 1, a similar significant improvement to long delay word recall 25 minutes after an acute BB treatment (143 mg anthocyanins) was observed by Whyte and Williams (2015). Both studies exhibited a greater number of words recalled after a delay. These findings further support the adult literature where Bell et al. (2018) observed a significant improvement to delayed word recall on the same AVLT task 1h30 after acute administration of a flavonoid-rich haskap berry drink (200 mg anthocyanins). In particular, healthy adults recalled a greater number of words 25 minutes after initial word list presentation following the 200 mg haskap treatment compared to placebo, and to a 400 mg haskap treatment. Although duration of recall delay (either two minutes after word list presentation for short delay recall or 25 minutes after word list presentation for long delayed recall) differed between these three studies, the findings collectively demonstrate that acute berry-flavonoid treatments can improve word list recall after a delay in both children and healthy adults. This suggests that acute berry-flavonoid treatments can aid the retention and retrieval of encoded words up to 25 minutes after initial presentation.

However, this improvement to delayed word recall was not observed following either acute or chronic WBB supplementation in Studies 2 and 3, even though both studies employed the same methodology, treatments, and test battery, used previously (Study 1). Due to the small sample recruited in Study 3 (N = 30, n = 15 per treatment group),

insufficient power may have contributed to the inability to detect a treatment effect on delayed word recall either acutely or chronically. This lack of power would not only affect delayed word recall but all cognitive measures, reading behaviour, and mood, assessed in Study 3. However, this same rationale cannot account for the lack of treatment effect for delayed word recall in Study 2. Indeed, it is important to note that, although a significant effect was observed for delayed word recall in Study 1, the effect size for this result was rather small (*d* = .076). As this treatment effect, along with one other (total word recall), were the only two word-list recall and recognition measures shown to significantly improve following acute WBB intake in Study 1, it is possible that these results may be due to type I error whereby multiple comparisons may lead to spurious findings; therefore, interpretation should be taken with caution.

The other treatment effect noted for episodic memory was the significant improvement to total word recall two hours after acute WBB consumption (Studies 1 & 2). This effect is more robust than that seen for delayed word recall two hours after WBB intake as was observed consistently in two studies within this thesis. Further, this supports Whyte et al. (2016) where a significant improvement in final acquisition was seen 75 minutes after intake of the same acute 30 g freeze-dried WBB powder treatment. The consistency of this finding highlights the potential for acute WBB treatments to improve immediate word learning in children (for a further discussion, see Section 6.2.2).

These significant improvements to total word recall and delayed word recall following acute WBB supplementation demonstrate a potential benefit of acute berry-flavonoid interventions on the immediate encoding and delayed retrieval of stimuli learnt over repeated exposure phases. Here, the encoding of words from a list learnt over five repeated recalls was significantly greater following acute WBB intake compared to a placebo; as a result of acute WBB intake directing greater levels of sustained attention to the stimuli, their encoding and later retrieval. This finding may potentially lead to a real-world benefit on learning in a school environment, such as when children are revising for exams. This suggests that an acute WBB treatment may potentially aid the encoding of information in general. Next, the present research demonstrates that acute WBB intake may improve the retrieval of learnt words up to 25 minutes after initial learning. This suggests the potential for an acute WBB treatment to aid the retention and retrieval of learnt information, such as in an exam setting or during school lessons.

In contrast to the previous literature in children, the present studies failed to observe either an acute or chronic effect of WBB supplementation on word recognition. Whyte et al. (2016) observed a general decline in word recognition performance over a six-hour period, however this decline was significantly greater for those consuming a placebo, compared to those consuming the 30 g freeze-dried WBB powder treatment (253 mg anthocyanins). Instead, the 30 g WBB treatment attenuated this decline in word recognition by maintaining performance over the six-hour period. One explanation for the present lack of WBB effects on word recognition is that improvements to word recognition may only be apparent at a later time after consumption. Indeed, research by Rodriguez-Mateos et al. (2013) helps explain this as BB flavonoid metabolites were shown to peak biphasically within the bloodstream at one to two hours and six hours after consumption of a flavonoid-rich treatment (766 mg total polyphenols). Therefore, differing effects of berry-flavonoids may be apparent at different time points after consumption. Whyte et al.'s (2016) findings support this as improvements to word recall final acquisition were only observed at 75 minutes after acute WBB intake whilst word recognition effects were only observed six hours after treatment. This suggests that word recognition effects from the acute WBB treatment may have been observed by the present studies if performance was assessed at a later test session, such as six hours after WBB consumption.

However, this potential explanation does not account for the significant improvement to word recognition observed 1h30 after consumption of the 200 mg anthocyanin and 400 mg anthocyanin haskap berry treatments in healthy adults (Bell et al., 2018). Further, Dodd et al. (2019) also demonstrated that intake of an acute 30.1 g flavonoid-rich BB treatment (579 mg anthocyanins) significantly improved word recognition two hours after treatment supplementation on a modified version of the Consortium to Establish a Registry for Alzheimer's Disease (Morris et al., 1989), a cognitive test battery of 14 tasks assessing executive function and memory. It is important to note that studies reporting berry-flavonoid effects on word recognition in adults and child differ in the berry-treatments used. First, the anthocyanin concentrations differ between the majority of these studies which may potentially contribute to the differences in word recognition findings. Indeed, both Bell et al. (2018) and Dodd et al. (2019) reported word recognition effects at 1h30 to two hours after consuming treatments containing high concentrations of anthocyanins (Bell et al., 2018: 400 mg anthocyanins; Dodd et al., 2019: 579 mg anthocyanins). These anthocyanin

concentrations were considerably higher than the 30 g freeze-dried WBB treatment (253 mg anthocyanins) employed by the present research (Studies 1-4) and Whyte et al. (2016), and the 100 ml BB treatment (143 mg anthocyanins) used by Whyte and Williams (2015). However, Bell et al. (2018) still observed a significant improvement to word recognition 1h30 after intake of the 200 mg anthocyanin haskap berry treatment. Of further importance here is the berry treatment used. Bell et al.'s (2018) haskap berry treatments may not only differ from the BB/WBB treatments in terms of anthocyanin concentration, but also in terms of other treatment constituents such as total polyphenol concentrations, vitamin/mineral concentrations, and fibre concentrations; all of which may contribute to the results. Therefore, the results obtained from Bell et al. (2018) can only be generalisable to other acute haskap berry studies, suggesting that comparisons to the acute BB/WBB studies need to be made with caution.

6.2.1.2 Executive function

The present studies demonstrate a consistent effect of significantly faster executive function reaction times following both acute and chronic WBB supplementation at times requiring greater cognitive demand: for the more cognitively demanding fast trials (Studies 1-3), and during afternoon testing (Study 2). These findings support the previous literature in children (Whyte et al., 2016; 2017). Whyte et al. (2016) observed significantly faster reaction times on the more cognitively demanding trials of a Picture Matching Task (Whyte et al., 2016) up to six hours after WBB intake. Similarly, Whyte et al. (2017) observed significantly faster executive function reaction times for more cognitively demanding incongruent, high load trials on the MANT three hours after acute WBB intake. Further, these findings are in line with the literature in healthy adults. Whyte et al. (2019) observed that healthy adults had significantly faster reaction times on the more cognitively demanding MANT trials four hours after intake of an acute 400 ml mixed berry treatment (254.65 mg anthocyanins).

Not only do the present results align with the previous executive function literature in children and adults, they extended these initial findings by observing a significant improvement to executive function at times of cognitive fatigue (Study 2). Acute WBB supplementation significantly improved reaction times for the more cognitively demanding trials when children were tested in the afternoon compared to those tested in the morning. Children tested in the afternoon would have experienced greater cognitive fatigue due to

the additional processing of information learnt throughout the day around the cognitive testing, in line with the morningness/eveningness chronotype at this age (Diaz-Morales et al., 2016; Vink et al., 2001; Adan et al., 2012). This additional information could have placed additional demand on the cognitive resources needed when completing the more difficult executive function trials, for example, in order to avoid interference effects or the inhibition of distracting stimuli. Although the influence of circadian timing and its importance for cognitive processes in adults is well established, relatively little is known about the homeostatic and circadian processes in children under the age of 9 years, and how these processed affect their cognition (Wright et al., 2012). Further, the continual developmental changes in brain structure and function associated with cognition in children of this age (Stiles & Jernigan, 2010) makes determining the effects of circadian rhythm on cognition more difficult to determine. In Study 2, subjective reports of fatigue were not assessed during this study so the potential effect of fatigue or circadian rhythm on performance, and whether acute WBB is effective at overcoming these effects, cannot be known for certain.

Acute WBB-related improvements on performance at times of heightened cognitive demand are supported by the literature in adults (Watson et al., 2015), where a maintenance of executive function reaction times under conditions of induced cognitive fatigue was demonstrated following an acute blackcurrant extract; the placebo showed a decline in performance over continuous repetitions of a cognitive test battery. In this instance, cognitive fatigue in participants was induced by the completion of continuous repetitions of an executive function task. Further support by Whyte et al. (2019) demonstrated significantly faster reaction times on a Trial Switching Task over a six-hour testing period; the greatest improvements were observed at later test sessions where participants experienced induced cognitive fatigue. Together, these findings suggest that acute berry-flavonoid treatments rich in anthocyanins can improve executive functions during times of high cognitive demand, such as for trials requiring greater cognitive resources and when these trials are completed later on in the test day under conditions of induced cognitive fatigue.

These significant improvements to executive function reaction time at times of heightened cognitive demand were also observed chronically (Study 3). This demonstrates that the benefits to cognitive performance observed at times of greater cognitive demand remain following prolonged WBB consumption. In addition, this significant improvement was

greater at four weeks compared to two weeks, suggesting that improvements increase and strengthen over a prolonged period of supplementation. Although the chronic berry-flavonoid literature in children is novel, the adult literature highlights chronic improvements to cognition over several months in healthy individuals (Miller et al., 2019) and in those with self-reported memory complaints (Whyte et al., 2018), as well as providing support for the accumulative WBB effects on cognition when consumed over a chronic supplementation period. Consuming a habitual diet high in berry-flavonoids from such a young age may have the potential to ameliorate the onset of cognitive complaints in aging older adults. Indeed, epidemiological studies demonstrate a positive association between high flavonoid intake and the delay in onset of cognitive aging. For example, Devore et al. (2010) found that older women (aged 70 years and above) who consumed one to two portions of blueberries and strawberries per week showed a delay in their cognitive aging equivalent of 1.5-2.5 years. This was in comparison to those who consumed one to two portions of berries per month. Therefore, the introduction of a habitual diet high in flavonoids in childhood may work towards preventing cognitive aging in later life. However, results from epidemiological studies should be interpreted with caution as it is often difficult to determine cause and effect. For example, those who consumed a greater portion of berries may have led an overall healthier life in terms of diet and exercise compared to those who consumed smaller quantities. Therefore, children who live a healthier lifestyle may well have a greater potential to ameliorate the onset of cognitive decline during later years, with greater dietary-flavonoid consumption being one contributing factor among many.

The consistent observation of WBB-related improvements to executive function reaction times under conditions of high cognitive demand can potentially benefit performance in the classroom. For example, providing school children with a mid-morning WBB snack may lead to greater sustained attention and an increase in the ability to inhibit interference effects of during lessons, particularly when taking place in the afternoon where alertness levels are known to decline (Cerasuolo et al., 2016). As the above WBB-related improvements have also been observed in healthy adults (Bell et al., 2018), a mid-morning WBB snack may have the potential to benefit performance in the workplace by overcoming the negative effects of cognitive fatigue experienced in the afternoon.

6.2.2 Reading behaviour

Acute-WBB improvements were observed for total word recall and delayed word recall (Studies 1 & 2) on a list-wise word learning task, suggesting that berry-flavonoids can benefit the encoding and retrieval of words on an episodic memory task. However, these acute and chronic benefits to list-wise word learning did not extend to word reading when assessed by the TOWRE-2. This null effect of acute WBB supplementation on word reading contrast to the previous literature examining the effects of PUFAs supplementation on reading in children. Johnson et al. (2017) observed significantly faster phonemic decoding time and visual analysis time following three-month omega-3 and omega-6 supplementation in healthy children. Further support from the PUFAs literature demonstrated (Milte et al., 2011; Zhang et al., 2006; Dalton et al., 2009; Richardson et al., 2012) significant word reading improvements to words and non-words when employing similar word reading tasks to the TOWRE-2.

When considering these previous improvements to word reading following PUFAs supplementation, the null effect of acute WBB consumption on reading behaviour observed by the present research was therefore unexpected. As the previous PUFAs literature focused specifically on chronic interventions and considering the positive effects of chronic WBB intake on the cognitive domains involved in reading, the chronic effects of WBB supplementation on reading behaviour were investigated (Study 3). However, chronic WBB supplementation did not affect word reading. As suggested above (Section 6.2.1.1), the small sample size (N = 30, n = 15 per treatment group) recruited in Study 3 may have contributed to this null effect where limited power was thought to contribute to the lack of chronic findings. Therefore, a larger sample size may yield more realistic findings for the effects of chronic WBB supplementation on reading behaviour.

A further explanation for the lack of acute and chronic WBB effects on reading behaviour was the specific task used. In general, the TOWRE-2 demonstrated that all children read aloud a greater number of words compared to non-words. This is consistent with theoretical frameworks of word reading such as the Dual Route Model of reading aloud (Coltheart et al., 1993) and triangle models (Harm & Seidenberg, 2004; Plaut et al., 1996; Seidenberg & McClelland, 1989) where known words are read at a quicker rate by automatic recognition through the lexical route (Dual Route Model) or the orthography-to-phonology pathway

(triangle models). The present research suggests that the TOWRE-2 may not be sensitive enough to detect subtle changes to word reading following acute and chronic doses of berry-flavonoids (Section 2.5). Unlike the BAS and WIAT-III word reading tasks used by the PUFA literature (Richardson et al., 2012; Milte et al., 2011) which assessed word reading without a time demand, the timed element of the TOWRE-2, where participants had to read as many words and/or non-words in 45 seconds, was thought to distract participants from correctly reading a known word or correctly decoding a non-word. This would have led to identification and decoding errors which would have negatively affected the fluency of children's word reading (Section 4.5). Although the TOWRE-2 is adept at measuring reading efficiency for the developmental literature, it is questionable whether the TOWRE-2 is sensitive enough to detect any WBB-related effects on decoding ability or sight word reading. This suggests the TOWRE-2 may not be the most appropriate measure of reading to use for dietary intervention studies.

The issue of task appropriateness of the TOWRE-2 was addressed by Study 4 which examined the effects of acute WBB supplementation on a different aspect of reading behaviour: learning to read words in story context, thus mimicking the natural process of how children learn to read novel words. Acute WBB intake did not affect learning to read words (orthographic learning) with no difference observed between immediate retrieval of newly learnt words two hours after acute WBB intake or delayed retrieval 24 hours later. This highlights a consistent null effect of WBB supplementation on word reading as assessed by the TOWRE-2 and orthographic learning, as well as demonstrating that acute WBB does not promote the delayed retrieval of learnt words. However, this contrasts to the consistent episodic memory findings (Studies 1 & 2), where significant improvements to delayed word recall, measured on a list-wise word learning task, were observed following acute WBB supplementation. At present the retention of acute WBB-related cognitive effects have not yet been examined over the 24-hour period, with delayed word retrieval effects only observed two hours after treatment. The bioavailability of a single dose of WBB treatment may not be sufficient enough at a 24-hour test point to affect word learning, or even cognition. In order to examine the retention of learning to read words over a 24-hour period, it would be beneficial to examine cognitive performance alongside this measure of word learning. As executive functions and episodic memory are known to be sensitive to the effects of acute WBB supplementation, examining cognition alongside learning to read

words would indicate whether the acute WBB treatment was having an effect on performance at the 24-hour test session. This would allow for an accurate conclusion to be made regarding the effects of acute WBB consumption on word learning over a 24-hour period.

Although the previous PUFAs literature observed improvements to reading behaviour, the majority of these improvements were observed in children performing below the normed range of age-matched peers, and in those with learning difficulties and developmental conditions (Richardson & Montgomery, 2005; Richardson et al., 2012). Therefore, comparisons between these studies and the present research should be taken with caution. All children recruited for the present research were high performing readers, allowing for the examination of WBB effects on reading behaviour in typical populations, before examining its potential to aid those with learning difficulties. Studies 1 to 3 noted that the YARC standard scores for all components: reading accuracy, reading rate, reading comprehension, and SWRT, for both treatment groups were between the mean and one standard deviation above the mean (M = 100, SD = 15). This indicated that the children recruited were recognised as high performing readers. Further, children recruited for Study 4 were subject to a screening session, whereby those who performed below the age-range on the TOWRE-2 and WASI-II, a measure of non-verbal intelligence, were excluded from the study. For this study it was important that children were able to decode correctly as low scores on these measures may have suggested poor performance on the word learning task employed in this experiment. Therefore, it is plausible that WBB may not benefit single word reading or word learning in those who are performing in line with or above their normed age range, but that the greatest benefits of WBB supplementation may be seen in those with initial poor performance. Indeed, Richardson et al. (2012) observed that children who performed 10-20% below that of age-matched peers at baseline showed a greater improvement in their reading after receiving the DHA treatment (Section 6.3.2).

The previous findings highlight that PUFAs interventions might only have an effect in children with lower habitual levels of PUFAs and thus may not affect children with normal dietary levels. For example, epidemiological research by Zhang et al. (2005) showed that children with below average reading performance on the Word Reading Achievement sub-test of the BAS had low habitual omega-3 levels. Further, research by Milte et al. (2011) observed that children with both ADHD and learning difficulties who had poorer word

reading performance had lower levels of omega-3 PUFAs compared to those performing at or above their age range who were not deficient in omega-3 PUFAs. Habitual flavonoid intake was not observed for the acute studies within this thesis, however habitual diet was assessed in Study 4. Here, the number of portions of fruit and vegetables was observed via food diaries, noting that on average children consumed between three to five portions of fruit and vegetables per day. Although this is not a clear indicator of overall habitual flavonoid intake, it can be assumed that the sample recruited were not deficit in flavonoids. This suggests that WBB may not affect those with a normal habitual flavonoid intake but cannot rule out the effect of berry-flavonoids in children with a deficient diet (Section 6.3.2). However, it is important to note that this measure of the number of fruit and vegetable portions consumed per day was only used to characterise the sample and was not included as a covariate within the analyses. Therefore, this interpretation of results should be considered with caution until this hypothesis can be analysed properly by future research.

6.2.3 Mood

Significant improvements to positive affect have been observed throughout this thesis. Acute effects of WBB supplementation demonstrated a clear significant improvement to self-reported positive affect scores two hours after treatment (Study 1). This finding is consistent with Khalid et al. (2017) where healthy adolescents reported improved positive affect two hours after an acute 30 g freeze-dried WBB treatment. Further, a maintenance of both positive affect and negative affect was observed following chronic WBB treatment over a four-week period (Study 3). Here, those consuming the placebo showed a significant decrease in positive affect at four weeks compared to two weeks, whilst also showing a significant increase in negative affect over the four-week period. Chronic WBB supplementation showed no significant difference in positive affect or negative affect over the four-weeks, maintaining self-reported affect following prolonged WBB consumption. Although neither acute nor chronic WBB supplementation reduced negative affect, suggesting that the emotions/feelings such as ‘sad,’ ‘disgusted’ and ‘anxious,’ associated with mood disorders were not reduced, they instead remained stable following acute and chronic WBB supplementation. These findings highlight a potential, natural intervention for improving and maintaining mood. However, it is possible that other confounding variables not measured by the study may have contributed to the adverse changes in mood observed in the placebo group which did not affect participants randomly allocated to the WBB group.

Further, this difference could be caused by participants with low mood in general, and therefore having lower mood at baseline, being randomly allocated to the placebo group. For this study, baseline scores for positive affect and negative affect significantly predicted later mood scores. Here, participants with greater affect scores at baseline had greater affect scores at the later test sessions.

At present, research examining the effects of WBB supplementation on mood is limited, with some studies reporting a null effect of treatment on mood (Whyte et al., 2018; Watson et al., 2015; Miller et al., 2019). As mentioned previously (Section 1.1.1.5), it is possible that the range of different mood scales used by the literature, which subsequently measure different aspects of mood (positive and negative affect, arousal, fatigue), could contribute to the inconsistent findings. Therefore, replication is needed using a standardised mood measure, such as the PANAS-C which measures affective state rather than arousal or fatigue, to clearly assess the acute and chronic effects of WBB consumption on mood (Section 6.3.3).

In general, the mood findings throughout this thesis occurred in parallel to significant improvements in executive function and episodic memory, highlighting a potential shared mechanism of action between mood and cognition. Indeed, Studies 1 and 3 observed significant improvements to cognition following both acute and chronic WBB supplementation, whilst further observing secondary improvements (Study 1) or a maintenance to positive affect (Study 4). This potentially suggests shared mechanisms of action which may underlie improvements to both cognition and mood following WBB intake. Indeed, this is supported by Watson et al. (2019) who observed improvements on a digit-span short-term memory task following acute blackcurrant juice. This cognitive finding was parallel to anxiolytic effects and the activation of mood related brain regions of interest whilst undergoing EEG. Research has started to examine the potential mechanisms of action underlying the effects of berry-flavonoids on cognitive performance, as well as mood, by completing cognitive tasks or mood measures at the same time as physiological measures e.g., fMRI to assess CBF (Bowtell et al., 2017).

However, improvements to cognition reported in this thesis were not always associated with improvements in positive affect. For example, the acute comparison study (Study 2) observed a significant interaction between time of day and WBB treatment on executive function reaction times, whilst no interaction was observed for mood. These findings suggest

that mood may have been the same in both the morning and afternoon, helping to explain the null effect of time of day and treatment for this study. As this was a comparison study, there is the potential for the masking of effects at the individual times of day. In order to examine this further, a separate analysis of the morning data set would be able to reveal the specific effects of acute WBB on mood when consumed in the morning.

Analysis of the combined data set observed no effects of acute WBB treatment when assessed independently. In this case treatment did not significantly predict either positive affect or negative affect. In contrast, overall greater positive affect was observed in the morning compared to the afternoon, regardless of treatment. Although the limited research thus far examining the effects of time of day on mood in adults posits greater positive affect in the afternoon (Diaz-Morales et al., 2016; Murray et al., 2002), morningness and eveningness chronotypes help to explain why the reverse is seen in children (Section 3.5). This does not nullify the effect of acute WBB consumption on mood, but rather demonstrates that further research is needed to clarify the inconsistent data thus far. Indeed, as stated in Section 6.2.1.2, research examining the influence of circadian rhythm on children's cognition is still novel so warrants further investigation before concrete conclusions can be made.

Research from this thesis suggests that mood effects remain after a chronic WBB treatment (Study 3). Although chronic WBB supplementation was observed to maintain positive affect over the four-week period, with no WBB-related improvements observed, the full effects of chronic WBB consumption on mood may be masked by the small sample size recruited for this study (Section 6.2.1.1). Therefore, a larger sample size generating greater power may show a greater effect of chronic WBB supplementation on mood, such as an increase in positive affect to align with the acute data (Study 1). Further, the sample size for this study was calculated using the WBB group's mean baseline performance on a measure of episodic memory total word recall rather than performance from a mood measure. Therefore, this study was not powered for mood, but rather for cognition, providing another explanation for the lack of chronic WBB effects on mood.

Overall, the mood effects from this thesis, along with the previous berry-flavonoid and mood literature, has started to demonstrate both acute and chronic effects of WBB intake on positive affect in both children (Studies 1 & 3) and adolescents (Khalid et al., 2017).

6.3 Future directions

This section details the suggested future directions of research when examining the acute and chronic effects of WBB supplementation on cognition, reading behaviour, and mood, based on the findings from this thesis.

6.3.1 Cognition

The acute WBB-related effects on total word list recall, short delayed word list recall, and executive function reaction times, observed by the present research are consistent with the previous literature, however the chronic effects of WBB supplementation on children's cognition are less clear. Although Study 3 observed limited effects of chronic WBB supplementation on cognition, mainly for executive function reaction time, the lack of cognitive findings was considered a consequence of the small sample size recruited leading to significant underpowering. Therefore, it would be prudent for future research to replicate this study, examining the effects of chronic WBB supplementation on children's cognition in a larger sample size. Indeed, an *a priori* power analysis based on the findings from Study 1 revealed a total of 42 children would be needed to provide sufficient power for the study, however due to slow recruitment, only 30 children took part (Section 3.5).

In general, future research should consider whether the improvements to episodic memory and executive function can benefit children's performance in the classroom. Indeed, acute WBB-related improvements to the encoding and retrieval of words assessed by a list-wise word learning task (Study 1) highlights the possibility that acute WBB intake may benefit the encoding and retrieval of information learnt in the classroom. This may have an additional benefit during revision as encoding of words learnt over repeat exposure phases was improved following acute WBB intake. Further, the consistent acute WBB-related improvements to executive function reaction times at times of greater cognitive demand: for example, on the more cognitively demanding trials or when children are tested in the afternoon, highlights the potential for a single, one-off dose of WBB to improve performance when children are experiencing heightened cognitive demand. Therefore, a WBB treatment given mid-morning may aid children's performance in the afternoon, two hours later. At this time of day children experience cognitive fatigue which may hinder their performance. A WBB treatment may therefore help children sustain greater levels of attention to overcome interference of distracting information and to overcome cognitive

fatigue effects. With this in mind, future research should consider whether the significant improvements observed on the cognitive test battery used by the previous research could improve school performance, compared to those who receive a no treatment control. As school and academic performance spans a vast majority of topics, for example: reading, arithmetic, it would be interesting to investigate whether WBB supplementation can improve performance on these real-world tasks, as well as any additional cognitive domains that these skills rely upon which have not been examined thus far. Indeed, multivitamin and mineral (MVM) interventions have been shown to significantly increase reading speed, learning capacity, arithmetic and non-verbal intelligence in healthy children (Benton et al., 2001; Schoenthaler et al., 2000; Wang et al., 2003). Further, habitual breakfast consumption in adolescents was more likely to result in higher mathematics GCSE grades compared to those who rarely consumed breakfast (Adolphus et al., 2019). To date, the effects of berry-flavonoid supplementation on academic performance in children has not been investigated, even though positive benefits have been consistently observed on some of the cognitive domains that underpin these skills: episodic memory, executive function, sustained attention, inhibition (Studies 1-3; Whyte and Williams, 2015; Whyte et al., 2016; 2017). Once this has been examined, research could examine the potential for acute WBB supplementation to further improve or maintain performance on these skills during times of cognitive fatigue, such as when performance is assessed in the afternoon.

As noted above (Section 6.2.1.1), there are many confounding variables to overcome in order to examine these hypotheses and to generalise the present findings to educational settings. One confounding variable to consider is plausibility of commercially available berries to benefit cognition, and therefore school performance. Indeed, the treatments used by research are not always commercially available to the general public. Further, berry fruits available in supermarkets are not consistent, and hence vary in a number of key constituents: anthocyanin concentrations, concentrations of total polyphenols, concentrations of fibre and other vitamins/minerals, all of which may contribute to changes in performance. This lack of a standardised, commercially available treatment highlights that the potential effects on performance will differ considerably amongst children, if apparent at all following such potential habitual intake. Future research should aim to determine a range of anthocyanin concentrations from commercially available berries, to include a minimum and maximum anthocyanin concentration where improvements to cognition are observed.

This would be needed to overcome the lack of control of differing anthocyanin concentrations found in different berries, or the same berry fruits from different origins or cultivated in different seasons. This would aid in generalising these improvements in cognition to school performance, and to the general public. Thus far, limited research has examined the dose-response effects of berry treatments with differing anthocyanin concentrations on cognitive performance (Whyte et al., 2016; Bell et al., 2018; Whyte et al., 2018; Watson et al., 2015), however this research has not considered the use of commercially available berry fruits. At present, this leap from the use of berry-flavonoid interventions in controlled experimental settings to an everyday intervention available to the general public might be too ahead of its time. Indeed, further research is first needed to build upon the existing literature examining the effects of berry-flavonoids on cognition in children, before examining whether these benefits to cognition can extend to other real world tasks, such as academic performance. Only then should the dissemination of findings to the general public be considered as an option for an 'everyday' intervention.

Last, in order to examine the effect of acute WBB intake on delayed retention of learnt words over the 24-hour period, it is first important to examine the cognitive effects of an acute WBB dose over this length of time. To date, this has not been examined. By observing the bioavailability of berry-flavonoids from a single WBB treatment over a 24-hour period on the cognitive domains known to be sensitive to acute WBB intake: episodic memory and executive function, this will help future research determine whether it is realistic to expect a WBB-related improvement to word learning 24 hours after consumption. However, this is not without its difficulties. Research examining of the bioavailability of berry-flavonoids after ingestion involves either examining concentrations of berry-flavonoid metabolites within the blood stream or the excretion of metabolites in urine (Del Rio et al., 2010). Both of these methods would require further considerations such as the collection, storage and analysis of samples, training for the researcher to complete the above, additional funding, to name a few. Further, parents of participants may not be willing to allow their child to be subject to blood samples or be willing to collect urine samples, likely to be collected on multiple occasions depending on the study design, thus leading to potential problems with recruitment and underpowering of the study. Neuroimaging techniques could also be considered as an option to examine the effects of berry-flavonoids of CBF and brain region activity whilst completing cognitive tasks. However, these too may not be appropriate for

child samples. For example, techniques such as fMRI would require children to remain motionless for lengthy periods of time, and the equipment used may cause adverse behaviours/emotions such as being unsure, apprehensive or scared which may affect the performance on the cognitive tasks. At present, there is little to no research examining the bioavailability of berry-flavonoids in children, with the majority of research recruiting adult samples (for a review, see Williamson et al., 2018). Indeed, metabolism in general varies greatly between adults and children, with data collected from adult samples not comparable to child samples where growth and development are known to affect their metabolism (Davies et al., 2000). Therefore, although it would be simpler for research to examine the behavioural outcomes of berry-flavonoids over a 24-hour period, further neurophysiological measures would be needed to examine the underlying mechanisms of action leading to these behavioural changes. This has been successfully investigated on numerous occasions by the adult literature (Dodd et al., 2019; Watson et al., 2019; Boesfplug et al., 2018; Bowtell et al., 2017) but has not been examined in children so far.

6.3.2 Reading Behaviour

The research within this thesis failed to observe acute or chronic effects of WBB supplementation on word reading or learning to read words. A number of theories have been put forward to explain this lack of findings: the word reading measure used, the lack of complexity of word reading, sample recruited, and treatment duration. First, research from Studies 1 to 3 suggested that word reading may not be complex enough to elicit improvements following WBB supplementation. Indeed, executive function improvements were only observed during periods of greater cognitive demand, therefore it would be practical to consider the effects of WBB consumption on reading comprehension; a more complex skill making use of many executive functions at once (Cartwright, 2009). Indeed, executive functions such as planning, working memory (Sesma et al., 2009; Locascio et al., 2010), inhibition (De Beni & Palladino, 2000), and processing speed (Christopher et al., 2012) are positively associated with reading comprehension. Christopher et al. (2012) found that processing speed was a significant predictor of fluency, a key component for sufficient reading comprehension, whilst De Beni and Palladino (2000) observed that poor comprehenders made a greater amount of intrusion errors, resulting in higher rates of irrelevant information being recalled after reading a passage. Therefore, these findings question whether it is realistic for a single one-off dose of WBB treatment to improve word

learning and reading comprehension. Future research may consider replicating the word learning research (Study 4) using the chronic methodology from Study 3 to examine the effects of chronic WBB consumption on word learning and reading comprehension. After correcting the methodological issues recognised from the previous chronic study, for example sample size, it would be interesting to observe whether the accumulative effects of berry-flavonoids following long term supplementation would benefit word learning and reading comprehension.

Further, future research should consider examining the effects of WBB supplementation on children who are underperforming or with learning difficulties. The previous literature demonstrated clear improvements to word reading following chronic PUFAs consumption for those who were initialling underperforming at baseline (Richardson et al., 2012). This suggests that children who are struggling with their word reading performance may experience the greatest benefit from dietary interventions. One approach for examining this hypothesis would be to investigate whether children's baseline reading ability affects their later performance, and whether WBB intake can provide a greater benefit to reading in children who are underperforming at baseline compared to children who are performing in line with the age norms. For the present research examining the effects of berry-flavonoid intake in typical children, baseline performance was added as a fixed effect covariate to control for any potential differences in baseline performance within the model. This is a valid approach when using linear models, commonly used by similar research (Whyte et al., 2018; Dodd et al., 2019; Bell et al., 2020; Barfoot et al., 2017) and is further in line with the use of covariates in regression models; a statistical approach which shares similar principles to LMM (Field, 2013). However, in order for future research to examine whether berry-flavonoid intake can exert a greater benefit to underperforming children, the use of an alternative LMM statistical analysis should be considered, including baseline performance as an independent variable fixed effect. This would allow examination of the direct effects of baseline performance on children's later performance and any potential interactions with treatment. This approach would also be advantage when examining the effects of berry-flavonoid intake in children with below average sustained attention, verbal and non-verbal intelligence. Here, children who are performing below age matched peers on cognitive tasks at baseline, may experience a greater benefit of berry-flavonoid intake on later cognitive performance. To examine the plausibility of the proposed statistical approach for analysing

the effects of berry-flavonoids on cognition and reading ability in children, a reanalysis of the episodic memory and reading behaviour data in Study 1 was conducted as an example. Here, baseline performance was removed as a covariate and was replaced as an independent variable fixed effect to examine the direct effect of baseline performance on later performance, and any subsequent interactions (see Appendix P).

Next, future research should examine whether WBB supplementation can improve performance in those starting to learn to decode. As suggested previously (Section 5.5), younger children in the partial alphabetic phase of reading development (Ehri, 1995) do not have a concrete understanding of grapheme-phoneme correspondence rules, thus limiting their ability to decode novel words. Therefore, WBB supplementation may have the potential to improve decoding ability in children transitioning from the partial alphabetic phase to the full alphabetic phase where sight word reading and efficient decoding become established. Indeed, Johnson et al., (2017) observed significantly faster decoding times following chronic PUFAs supplementation in children, whilst the present study observed significant improvements to executive function reaction times for the more cognitively demanding trials following acute and chronic WBB supplementation. These findings highlight the potential for WBB consumption to improve decoding ability in younger children learning to decode.

Last, it would be interesting for future research to examine the effect of WBB consumption on reading behaviour in those with a low habitual intake of flavonoids, and whether additional flavonoid supplementation can improve performance. Indeed, epidemiological research by Zhang et al., (2005) noted that low omega-3 levels were associated with lower than average reading performance as assessed by the Word Reading Achievement sub-test of the British Ability Scale.

6.3.3 Mood

Research examining the effects of berry-flavonoids on mood is novel, and the studies detailed in this thesis are the first to observe the effects of acute and chronic WBB supplementation on mood in children. Thus far, the limited research demonstrated improvements to positive affect following acute WBB intake (Study 1) and a maintenance of positive affect following chronic WBB consumption (Study 3). Due to the inconsistency in mood effects reported in the limited literature (Watson et al., 2019; Whyte et al., 2018;

Miller et al., 2019; Watson et al., 2015), it is first important to replicate the acute and chronic findings in children to gain an understanding of the full effects of WBB supplementation on mood when using a standardised measure. Once this has been conducted, future research should consider examining the potential shared underlying mechanisms of action between cognition and mood. Indeed, it is apparent that mood effects can coincide with cognitive effects (Watson et al., 2019; Studies 1 & 3), with WBB treatments able to utilise these potential shared mechanisms of action to improve mood and cognition. Physiological measures have started to be used in the cognitive literature, for example fMRI to measure CBF (Bowtell et al., 2017), therefore such techniques should be considered when examining the underlying mechanisms of action for berry-flavonoid mood effects as well, however, see Section 6.3.1 for the difficulties of using these techniques in child samples.

Further, future research should consider whether WBB supplementations can benefit those susceptible to low mood. As acute WBB supplementation has been shown to improve positive affect in children without depressive symptoms, the findings highlight the potential for WBB intake to improve positive affect in those with low mood. In addition, the maintenance of positive and negative affect following chronic WBB highlights the potential for the accumulative effects of prolonged berry-flavonoid supplementation on those with low mood.

6.4 Final conclusions

This series of WBB studies observed significant effects of acute WBB supplementation on episodic memory word list recall and delayed word list recall. These findings suggest that acute WBB intake can potentially aid the encoding and retrieval of learnt words two hours after treatment, and the retention and retrieval of learnt words 25 minutes after a short delay. These findings highlight that the effects of acute WBB supplementation may benefit children in real world scenarios, for example in an everyday classroom setting. Here, acute WBB may aid the encoding of information learnt within the classroom, as well as aiding the retention and later retrieval of this information such as during an examination.

Further, acute and chronic WBB intake significantly improved executive function reaction times specifically for the more cognitively demanding trials. These findings suggest that acute and chronic WBB supplementation may benefit performance on other, more cognitively demanding tasks such as academic performance for arithmetic, maths and

reading. As research already highlights an association between executive function and academic performance (Section 1.2.5), it is possible that the benefits of berry-flavonoid supplementation observed for executive function, may extend to academic performance. Further, benefits to executive function were also observed at times of high cognitive demand such as when children were tested in the afternoon. These cognitive findings highlight the potential for WBB supplementation to benefit children when experiencing cognitive fatigue, such as in the afternoon when alertness levels start to decline. The cognitive findings highlight that future research should consider the dissemination of findings from controlled, experimental settings to more realistic, educational scenarios such as highlighted above.

In this instance, neither acute nor chronic WBB supplementation benefitted word reading or the learning to read words. It is possible that these aspects of reading are too simplistic for berry-flavonoids to improve upon. Therefore, in line with the cognitive improvements observed at times of greater cognitive demand, WBB supplementation may benefit reading comprehension; a skill set involving aspects of executive function known to be sensitive to WBB interventions. Further, as noted by the previous PUFAs literature, it is possible that WBB consumption may only improve reading for children performing below the normed range for their age group (Richardson et al., 2012), and in those with a low habitual flavonoid intake; in line with the previous PUFAs literature (Zhang et al., 2005). Therefore, although WBB supplementation did not benefit reading behaviour in children performing in line age norms, future research should consider the effects of WBB supplementation on both cognition and reading behaviour in underperforming individuals where WBB supplementation may exert its greatest benefits, as well as the effects of WBB supplementation in children with a poor diet.

Last, acute WBB supplementation significantly improved self-reported positive affect two hours after treatment suggesting that a one-off dose of WBB can potentially overcome the initial onset of low mood. Chronic WBB supplementation maintained positive affect over the four-week period, compared to placebo consumption which exhibited a decrease in positive affect and an increase in negative affect over the four-week period. These findings suggest that the accumulative effects of berry-flavonoid following prolonged WBB consumption can potentially provide a natural dietary intervention for overcoming low mood on a long-term basis.

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Appendices

Appendix A – Ethical approval for Chapters 2-5

A.1 UREC Ethics committee approval for Chapters 2-4.

From: Mike Proven

Sent: 13 March 2015 18:13

To:

Cc: John Wright ; Laurie Butler

Subject: UREC 15/10: The effects of wild blueberry supplementation on the behaviour and cognition of 7-10 year old participants with and without attention-deficit hyperactivity disorder (ADHD). Favourable opinion

Dear Claire

UREC 15/10: The effects of wild blueberry supplementation on the behaviour and cognition of 7-10 year old participants with and without attention-deficit hyperactivity disorder (ADHD).

Favourable opinion Thank you for the response (email dated 26 February 2015, including attachments refers) addressing the issues raised by the UREC Sub-committee at its February meeting. On the basis of these responses and the revised documentation, I can confirm that the Chair is pleased to confirm a favourable ethical opinion. Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion. Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practise in Research.

Yours sincerely

Dr M J Proven Coordinator for Quality Assurance in Research (UREC Secretary) cc: Dr John Wright (Chair); Dr Laurie Butler, Head of School

From: Doug Saddy

Sent: 22 August 2017 18:57

To: ;

Subject: Ethics application 2017-105-CW

Dear Claire, I have reviewed your application 2017-105-CW and find that it conforms to our school's ethical standards. Green light.

Best

Professor Douglas Saddy

A.2 Amendment to ethics for Chapter 5.

From: Gabrielle May

Sent: 26 September 2017 12:39

To: Liz White

Cc:

Subject: Amendment to ethics

Hi Liz,

I would like to request an amendment to the protocol of the attached study: The effects of wild blueberry supplementation on word learning in 8-9 year olds. Ethical approval was granted on 23/08 and marked 2017-1055-CW. Participant recruitment and data collection have started.

I would like to remove the following sentence from section 2.2 Procedure of my previously approved ethics application:

"Parents will also be contacted here if their child has scored below the average performance range for non-verbal IQ and TOWRE and it will be explained that, due to the likelihood of the child struggling with this task and potentially cause them distress, their child will discontinue participation in the study."

I no longer feel that it is appropriate to contact the parents of children excluded on this basis. By contacting parents and alerting them to the fact that their child has below average performance and will therefore potentially struggle completing the tasks, this message may lead parents to feeling their child needs additional support and resources. As researchers, we are not set up to deliver such a message which could lead to further implications for both the school and its resources. These tests used are for research purposes only and are not being used in a clinical or diagnostic capacity.

Instead, I propose that for the situation where children are excluded for below average performance, the parents are not contacted. These children will be allowed to complete the screening session and will be rewarded for their participation with a certificate. I have amended the ethics application to include:

"Those excluded for performing below the average range on the WASI non-verbal IQ and TOWRE will be given a certificate and thanked for their participation." - seventh paragraph, section 2.2 Procedure.

Kind regards,

Gabrielle May

From: Doug Saddy

Sent: 27 September 2017 15:48

To: ;

Subject: Amendment to ethics 2017-105-CW

I am happy to approve this amendment.

best regards

Professor Douglas Sady

Appendix B – Parent/legal guardian information sheets.

B.1 Parent/legal guardian information sheet for Chapters 2 & 3

Title of studies: Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Information Sheet

Supervisors: **Email:** **Telephone:**

Prof Claire Williams

Researcher:

Gabrielle May

I hope to provide all the information you will need about this study in order for you to make an informed decision about whether you would like your child to participate. However, if you have any further queries or would like to discuss any aspect of the study, please do not hesitate to contact me by email: .

Background to the study

We are interested in finding out about the effects of a fruit drink on the attention, memory and reading ability in 7 to 10 year old children without reading difficulties or developmental disorders such as ADHD. Some fruits naturally contain high amounts of micronutrients called flavonoids that are found in a number of foods including vegetables, fruits, and fruit juices. Recent research with adults has shown that consumption of foods with a high flavonoid content leads to beneficial improvements on such things as attention and memory. A recent study carried out within our department has shown that following consumption of a blueberry drink, children aged 7 to 10 years showed improved attention and memory performance. These results have academic and educational implications for children, such as potentially improving their reading.

Who is running the study?

The study is run by research staff in the School of Psychology & Clinical Language Sciences at the University of Reading. The researcher on this project has been through the formal Disclosure & Barring Service (DBS) procedure and has been approved by the University of

Reading to work with children. The study has been reviewed by the University Research Ethics Committee and has been given favourable ethical approval which means that an independent group did not raise any objections to the study on ethical grounds and have permitted the study to proceed. The study is funded by the University of Reading and a non-commercial nutritional supplement company. The study will take place throughout the remainder of the autumn term.

What does the study involve?

Practise Session

This will involve your child being taken out of the classroom at a convenient time in the school day to have a 'practise' of all the cognitive tasks that they will encounter at each future test phase. This is so your child can get used to the tasks and ask questions if they are unsure of what to do. This session will last approximately 1 hour.

Session 1

You will be asked to implement a low-flavonoid diet 24 hours before this Session. Details of what this entails are provided in this pack. If your child usually has school lunches then the researcher will ensure your child receives a low-flavonoid lunch from the cafeteria.

At the start of the school day, your child will then take part in the previously practised cognitive task battery and an additional reading task taking approximately 40 minutes. Your child will then receive a fruit drink to consume and will then re-join their fellow classmates to continue their school day as normal.

Session 2

This Session will take place 2 hours after Session 1. Your child will be taken out of the classroom and will be asked to perform the same cognitive task battery and reading task as before, taking approximately 40 minutes. A small token will be rewarded to your child at the end, e.g. a sticker chart, and they will then continue with the school day as normal. We will liaise with the school to minimise any disruption to your child's learning where possible.

About the fruit drinks…

Your child will receive one of two different doses of fruit drinks. The dose received will be randomly allocated. The two drinks will contain organic orange squash and a different dose of flavonoid. One of the drinks will also have a small amount of sugar and vitamin-C to match the amount found in the flavonoid drink. All drinks will be prepared hygienically within the University or school, and with the exception of the ingredients already listed, no additives or any other items will be added. A full breakdown of the ingredients can also be made available to you upon request. In order to ensure that it is truly the drinks that are having an effect, we will provide you with a list of foods that are high in flavonoid content, which we will ask your child to avoid eating for 24 hours before each study day.

The sessions are designed to be fun and consist of a few short tasks to do on a computer. One of the tasks will require your child to press arrow keys in accordance with different arrows displayed on-screen. The other task will require your child to listen to a list of words being read out via an audio recording, and then recall as many as possible, in any order. The reading task will require your child to read as many words and made-up words within a short time period.

Why has my child been selected to take part?

We have invited children from local schools aged 7-10 years old to take part in our study. We have targeted children of this age range as they are old enough to understand the tasks. Additionally, around the ages of 7 to 10 children experience a spurt of brain growth in areas related to attention and memory that makes their responses to these tasks of particular interest.

What happens to the data?

All information collected will remain fully confidential and no results from any of the tasks your child performs will be shared with your child's school. All the information you provide us with will be assigned an anonymous number and no name will appear on any of the documents. All data will be kept safely locked at the University of Reading and only the named researcher at the top of this sheet will have access. The data will be used only for research purposes, and in accordance with the Data Protection Act of 1998, they will be destroyed 5 years after the completion of the study.

On completion of this study there is a possibility that the results will be published in an academic journal. Only the overall results will be referred to in this publication with no direct reference being made regarding your child or the school they attend. Additionally, if you so request, the results of the study will be forwarded to you upon its completion either by post or email.

Does my child have to participate?

No. Participation in this study is entirely voluntary and you are under no obligation to agree to participate. Also, you may withdraw your child at any point during the study without giving any reason.

I'd like my child to participate, what happens next?

I am the researcher on this project and will be the initial contact for any queries throughout the study, so please do not hesitate to get hold of me via email. However, if by some means you are unable to contact me at any point, then you may also contact Professor Claire Williams (the Principle Investigator and my supervisor;) and she will be happy to answer your queries and to pass on any information to me.

B.2 Parent/legal guardian information sheet for Chapter 4.

Title of study: Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Information Sheet

Supervisors: **Email:** **Telephone:**

Prof Claire Williams

Researcher:

Gabrielle May

I hope to provide all the information you will need about the study in order for you to make an informed decision about whether you would like your child to participate. However, if you have any further queries or would like to discuss any aspect of the study, please do not hesitate to contact me by email: .

Background to the study

I am interested in finding out about the effects of a fruit drink on the attention, memory, and reading ability, of healthy school-aged children. Some fruits naturally contain high amounts of micronutrients called flavonoids that are found in a number of foods including vegetables, fruits and fruit juices. Recent research with adults has shown that consumption of foods with a high flavonoid content leads to beneficial improvements on such things as attention and memory. A recent study carried out within our department has shown that following consumption of a blueberry drink, children aged 7-10 years showed improved attention and memory performance. These results have positive educational implications for children, such as the possibility to benefit reading performance which relies on both memory and attention.

Who is running the study?

The study is run by research staff in the School of Psychology & Clinical Language Sciences at the University of Reading and the Department of Psychology at Royal Holloway, University of London. The researcher on this project has been through the formal Disclosure & Barring Service (DBS) procedure and has been approved by the University of Reading to work with

children. The study has been reviewed by the University Research Ethics Committee at the University of Reading and has been given favourable ethical approval; which means that an independent group did not raise any objections to the study on ethical grounds and have permitted the study to proceed. The study is funded by the University of Reading and a non-commercial nutritional supplement company.

What does the study involve?

Session 1

You and your child will meet with the researcher one week before the study and will be briefed on what the study entails. Your child will be asked to taste the fruit drink randomly allocated to them, and to rate its pleasantness from 1 – 10. Your child will then be asked to complete several cognitive, behavioural and demographic tasks. You will also be asked to complete a food questionnaire before the next session.

If your child really does not like the drink, shows distress from the taste, or as a result cannot finish the contents, then he/she will not be invited to participate any further.

Session 2

You will meet with the researcher one day before the study. Your child will be asked to complete a 'practise' of all the cognitive tests that they will encounter at each future test session. This is so your child can get used to the tasks and ask questions if they are unsure of what to do.

Prior to all future sessions, your child will be asked to follow a low-flavonoid diet for 24 hours beforehand. This is so that any effects observed can be related to the drink and not any other sources of high-flavonoids within their diet. Foods to avoid will be:

- All fruit and vegetables (except bananas, carrots and sweetcorn)
- Chocolate
- Fruit juice
- Tea and coffee
- Fizzy and energy drinks
- Pain relievers (e.g. paracetamol, ibuprofen, aspirin)

Session 3 – weekend home visit

This session will take place at your home on a Saturday and after following the 24 hours low-flavonoid diet. Your child will take part in the previously practised cognitive test battery and reading task taking approximately 45 minutes. Your child will then receive their fruit drink to consume and you will meet with the researcher 2 hours later for further testing. Your child must not eat anything in this 2-hour time period but may drink water.

Session 4 – weekend home visit

This session will take place on the same day as session 3, 2 hours later. Your child will perform the same cognitive test battery and reading task as before, taking approximately 45 minutes.

4-week fruit drink administration

After session 4, you will then be given 2 weeks worth of the fruit drink formula with instructions on how to prepare it. You will be asked to administer your child's drink every day for 4 weeks. Your child must finish all contents each day. In the event that they do not drink it one day, you must try to administer another (fresh) drink that day. If your child still does not comply, you should not administer the drink that day and record it as a 'missed day'. You will also be given a 3-day food diary to complete over the next 2 weeks. Dates and times for future sessions will also be provisionally booked during this session.

Session 5

This session will take place two weeks after session 4. For this session, you will be asked to follow the low-flavonoid diet 24 hours beforehand and you will also be asked to not give your child their fruit drink that morning. Your child will then complete the same test battery as before. You will then be given another 2 weeks' worth of the fruit drink formula to continue administration every day, and will be given a 3-day food diary to complete, as before, over the next 2 weeks.

Session 6 – no drink to be consumed for this day

This session will take place two weeks after session 5. The procedure of this session will be identical to that session 5, except children will not receive a drink after testing. You will then both be debriefed about the experiment and any questions will be answered. Any remaining

fruit drink formula will be collected back from you.

About the fruit drinks…

Your child will receive one of two different fruit drinks. The drink received will be randomly allocated. The two drinks will contain organic orange squash and a different dose of flavonoid. One of the drinks will also have a small amount of sugar and vitamin-C to match the amount found in the flavonoid drink. All drinks will be prepared hygienically within the university or school, and with the exception of the ingredients already listed, no additives or any other items will be added. A full breakdown of the ingredients can also be made available to you upon request. In order to ensure that it is truly the drinks that are having an effect, we will provide you with a list of foods that are high in flavonoid content which we will ask your child to avoid eating for 24 hours before each study day. A list of optional healthy alternative food will also be provided. Full details of this will be made available to you in advance.

The sessions are designed to be fun and consist of a few short tasks to do on a computer. One of the tasks will require your child to follow sets of arrows and they will have to press a key indicating the arrows' directions. The other task will require your child to listen to a list of words being read out via an audio recording, and then recall as many as possible, in any order. The reading task will see how many words and pronounceable nonwords your child can read in 45 seconds.

What happens to the data?

All information collected will remain fully confidential and no results from any of the tasks your child performs will be shared with your child's school. All the information you provide us with will be assigned an anonymous number and no name will appear on any of the documents. All data will be kept safely locked at the University of Reading and only the named researcher at the top of this sheet will have access. The data will be used only for research purposes, and in accordance with the Data Protection Act of 1998, they will be destroyed 5 years after the completion of the study.

On completion of this study there is a possibility that that the results will be published in an academic journal. Only the overall results will be referred to in this publication with no direct reference being made regarding your child or the school they attend. Additionally, if

you so request, the results of the study will be forwarded to you upon its completion either by post or email.

Does my child have to participate?

No. Participation in this study is entirely voluntary and you are under no obligation to agree to participate. Also, you may withdraw your child at any point during the study without giving any reason.

Will my child be paid to participate?

You and your child will not be paid for participation, however, your child will be given a T-shirt as a token of our appreciation upon completion of the whole study.

I'd like my child to participate, what happens next?

Please contact me, Gabrielle May, **via e-mail** (indicating the information below, **or** tear off the slip below and **send to:** Gabrielle May, Room 201, School of Psychology and Clinical Language Sciences, Earley Gate, University of Reading, Whiteknights Road, Reading, RG6 6AL. A member of the research team will then contact you to arrange the study visits and to answer any further questions you may have.

I am the researcher on this project and will be the initial contact for any queries or questions throughout the study, so please do not hesitate to get hold of me via email. However, if by some means you are unable to contact me at any point, then you may also contact Professor Claire Williams (the Principle Investigator and my supervisor;) and she will be happy to answer your queries and to pass on any information to me.

B.3 Parent/legal guardian information sheet for Chapter 5.

Title of study: Effects of acute wild blueberry supplementation on word learning in eight to nine year olds.

Information Sheet

Supervisors: **Email:** **Telephone:**

Prof Claire Williams **0118 378 7540**

Researcher:

Gabrielle May

We hope to provide all the information you will need about the study in order for you to make an informed decision about whether you would like your child to participate. However, if you have any further queries or would like to discuss any aspect of the study, please do not hesitate to contact me by email: .

Background to the study

We are interested in finding out about the effects of a fruit drink on children's ability to learn new words. Some fruits naturally contain high amounts of micronutrients called flavonoids that are found in a number of foods including vegetables, fruits and fruit juices. Research with adults has shown that consumption of foods with a high flavonoid content leads to beneficial improvements on such things as attention and memory, both involved in reading. Recently, research from the University of Reading has found similar improvements in attention and memory performance in 7-10 year olds after consuming a flavonoid-rich fruit drink. We now aim to examine whether these results have academic and educational implications for children by investigating the effects of a flavonoid-rich fruit drink on children's ability to learn new words when reading.

Who is running the study?

The study is run by research staff in the School of Psychology & Clinical Language Sciences at the University of Reading. The researcher on this project has been through the formal Disclosure & Barring Service (DBS) procedure and has been approved by the University of Reading to work with children. The study has been reviewed by the University Research

Ethics Committee and has been given favourable ethical approval which means that an independent group did not raise any objections to the study on ethical grounds and have permitted the study to proceed. Full agreement from (the school) to participate in this research has also been granted. The study is funded by the University of Reading and a non-commercial nutritional supplement company.

What does the study involve?

Screening Session

The screening session will involve your child being taken out of the classroom at a convenient time in the school day to complete some cognitive and reading tasks: IQ, general reading ability, and vocabulary. This session will last approximately 45 minutes.

Session 1

You will be asked to implement a low-flavonoid diet 24 hours before this session. Details of what this entails are provided in this pack. If your child usually has school lunches then the researcher will ensure your child receives a low-flavonoid lunch from the cafeteria.

In the morning, your child will meet the researcher before class to consume their fruit drink. They will then re-join their fellow classmates to continue the school day as normal. Your child will be taken out of the classroom 2 hours after consuming their drink to take part in some reading tasks: story reading and word learning. This session will last approximately 45 minutes.

Session 2

This session will take place 24 hours after session 1. You will again be asked to implement the low-flavonoid diet 24 hours before this session. Your child will be taken out of the classroom and will be asked to take part in the word learning tasks as before, taking approximately 20 minutes. A small token will be rewarded to your child at the end, e.g. a sticker. Following session 2, your child will return to class as normal. We will liaise with the school to minimise any disruption to your child's learning where possible.

About the fruit drinks…

Your child will receive one of two different doses of fruit drinks. The dose received will be randomly allocated. The two drinks will contain organic orange squash and a different dose of flavonoid. One of the drinks will also have a small amount of sugar and vitamin c to match the amount found in the high flavonoid drink. All drinks will be prepared hygienically within the university or school, and with the exception of the ingredients already listed, no additives or any other items will be added. A full breakdown of the ingredients can also be made available to you upon request. In order to ensure that it is truly the drinks that are having an effect, we will provide you with a list of foods that are high in flavonoid content, which we will ask your child to avoid eating for 24 hours before each study day.

The sessions are designed to be fun and consist of a few short tasks to do on a computer. One of the tasks will require your child to read some short stories about some new inventions. The other two tasks will involve your child learning some novel words.

Why has my child been selected to take part?

We have invited children from local schools aged 8-9 years old to take part in our study. We have targeted children of this age group as they are old enough to understand the tasks. At this age, children's reading abilities have become established, meaning they should have no difficulties with any of the reading tasks. Additionally, around the ages of 7 and 10 children experience a spurt of brain growth in areas related to attention and memory that makes their responses to these tasks of particular interest.

What happens to the data?

All information collected will remain fully confidential and no results from any of the tasks your child performs will be shared with your child's school. All the information you provide us with will be assigned an anonymous number and no name will appear on any of the documents. All data will be kept safely locked at the University of Reading and only the named researcher at the top of this sheet will have access. The data will be used only for research purposes, and in accordance with the Data Protection Act of 1998, they will be destroyed 5 years after the completion of the study.

On completion of this study there is a possibility that that the results will be published in an academic journal. Only the overall results will be referred to in this publication with no direct

reference being made regarding your child or the school they attend. Additionally, if you so request, the results of the study will be forwarded to you upon its completion either by post or email.

Does my child have to participate?

No. Participation in this study is entirely voluntary and you are under no obligation to agree to participate. Also, you may withdraw your child at any point during the study without giving any reason.

I'd like my child to participate, what happens next?

Please complete the attached consent form and return to (the school) office as soon as possible.

I am the researcher on this project and will be the initial contact for any queries throughout the study, so please do not hesitate to get hold of me via email: . However, if by some means you are unable to contact me at any point, then you may also contact Professor Claire Williams (the Principle Investigator and my supervisor;) and she will be happy to answer your queries and to pass on any information to me.

Appendix C – Parent/legal guardian consent forms.

C.1 Parent/legal guardian consent form for Chapters 2 & 3

Title of studies: Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Consent Form

I ……parent/guardian of ……..

agree to my child participating in the study 'The effects of fruit drinks on the cognition and reading ability of 7-10 year old children' run by the School of Psychology & Clinical Language Sciences, University of Reading at Thameside Primary School. This study has been reviewed by the University of Reading Research Ethics Committee and has been given ethical approval.

☐ I have seen and read a copy of the Study Information Sheet and have been given the opportunity to ask questions about the study via email and these have been answered to my satisfaction.

☐ I understand that all personal information will remain confidential to the researcher and arrangements for the storage and eventual disposal of any identifiable material have been made clear to me.

☐ The contents of the drinks have been explained to me and I am happy for my child to consume them.

☐ I understand that participation in this study is voluntary and that I can withdraw my child at any time without having to give an explanation.

☐ I believe that my child understands what is required of them during the study.

☐ I understand that I will need to implement a low-flavonoid diet for my child 24 hours before the study.

☐ I am happy for my child to proceed with participation.

Name (in capitals): ……..

Date:…….. Signature: ……..

Child's name: ……..... Child's DOB:……..

Child's age:…… Year and Class:……..

Does your child have school dinners? Y / N

Any reading difficulties/developmental diagnoses: ……

Medication: ……..

Email address:……

Phone number:……..

C.2 Parent/legal guardian consent form for Chapter 4.

Title of study: Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Consent Form

I ……parent/guardian of ……

agree to my child participating in the study 'The effects of fruit juice drinks on cognition and reading ability in 7-10 year olds at the School of Psychology & Clinical Language Sciences, University of Reading. This study has been reviewed by the University of Reading Research Ethics Committee and has been given ethical approval.

☐ I have seen and read a copy of the Study Information Sheet and have been given the opportunity to ask questions about the study and these have been answered to my satisfaction.

☐ I understand that all personal information will remain confidential to the researcher and arrangements for the storage and eventual disposal of any identifiable material have been made clear to me.

☐ The contents of the drinks have been explained to me and I am happy for my child to consume them.

☐ I understand that participation in this study is voluntary and that I can withdraw my child at any time without having to give an explanation.

☐ I believe that my child understands what is required of them during the study.

☐ I am happy for my child to proceed with participation.

Signature ……

Name (in capitals) ……

Date ……

C.3 Parent/legal guardian consent form for Chapter 5.

Title of study: Effects of acute wild blueberry supplementation on word learning in eight to nine year olds.

Consent Form

I ……parent/guardian of ……

agree to my child participating in the study 'The effects of fruit drinks on word learning in 8-9 year olds' run by the School of Psychology & Clinical Language Sciences, University of Reading. This study has been reviewed by the University of Reading Research Ethics Committee and has been given ethical approval.

□ I have seen and read a copy of the Study Information Sheet and have been given the opportunity to ask questions about the study via email and these have been answered to my satisfaction.

□ I understand that all personal information will remain confidential to the researcher and arrangements for the storage and eventual disposal of any identifiable material have been made clear to me.

□ The contents of the drinks have been explained to me and I am happy for my child to consume them.

□ I understand that my child will be required to follow a special low flavonoid diet for the duration of the study (48hrs) and have received the diet sheet detailing the foods to be avoided.

□ I understand that participation in this study is voluntary and that I can withdraw my child at any time without having to give an explanation.

□ I believe that my child understands what is required of them during the study.

□ I am happy for my child to proceed with participation.

Name (in capitals): ……..

Date:…….. Signature: ……..

Child's name: ……..... Child's DOB:……..

Child's age:…… Year and Class:……..

Does your child have school dinners? Y / N

Any reading difficulties/developmental diagnoses: ……

Medication: ……..

Email address:……..

Phone number:……..

Appendix D – Participant information sheets.

D.1 Participant information sheet for Chapters 2 & 3.

Title of studies: Effects of acute wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Information sheet

In this experiment we are trying to find out whether a fruit drink helps you do better when playing games which need you to use your memory and attention.

What will happen?

We will take you out of your classroom to play some games one day in the next few weeks. Some of the games will be played on a computer and some you will write down on paper.

Your mum or dad will give you a special breakfast and lunch on this day, and a special dinner the night before. After you have played some games with us, you will be asked to drink a fruit drink! You will then come back to play more games 2 hours later.

You will get the chance to have a practise of the computer games in the days leading up to your session, so please ask if you are unsure of anything then!

We will keep the scores of how well you do so we can find out if the drink makes you better at playing the games.

We won't tell anyone else about what you say or do in the experiments, but it is okay for you to tell other people if you want to.

If you don't want to have the drink or take part then you don't have to and you don't need to say why. When you have been taken out of lesson, if you decide you don't want to continue playing the games then you can stop at any time and you don't need to say why.

I hope that you will want to take part and be a scientist helper!

From, Gabbie (the scientist)

D.2 Participant information sheet for Chapter 4.

Title of study: Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Information sheet

In this experiment we are trying to find out whether a fruit drink helps you do better when playing games which need you to use your memory and attention.

What will happen?

The researcher will visit you six times at home or at the University to play some games on a computer and on paper. These games will need you to use your memory and attention. You will get the chance to have a practise of the computer games so please ask if you are unsure of anything then!

On one of the researcher's first visits, you will also be asked to drink a fruit drink after playing some of the games. You will then play more games 2 hours later.

After this visit you will be asked to drink the fruit drink every day for four weeks, you can help your mum and dad make the drink each morning. You will play the games again at two weeks and four weeks.

Your mum or dad will give you a special breakfast and lunch before each visit, and a special dinner the night before each visit.

We will keep the scores of how well you do so we can find out if the drink makes you better at playing the games.

We won't tell anyone else about what you say or do in the experiments, but it is okay for you to tell other people if you want to.

If you don't want to have the drink or take part then you don't have to and you don't need to say why. When you have been taken out of lesson, if you decide you don't want to continue playing the games then you can stop at any time and you don't need to say why.

I hope that you will want to take part and be a scientist helper!

From, Gabbie (the scientist)

D.3 Participant information sheet for Chapter 5.

Title of study: Effects of acute wild blueberry supplementation on word learning in eight to nine year olds.

Information sheet

In this experiment we are trying to find out whether a fruit drink helps you to learn some new words.

What will happen?

We will take you out of your classroom to play some reading games with you one day in the next few weeks. Some of the games will be played on a computer and some you will write down on paper.

Your mum or dad will give you a special breakfast and lunch on this day, and a special dinner the night before. Before you play the games with us, you will be asked to drink a fruit drink! You will then come back to play the games 2 hours later.

You will be able to ask the researcher any questions you have during the study.

We will keep the scores of how well you do so we can find out if the drink makes you better at playing the games.

We won't tell anyone else about what you say or do in the experiments, but it is okay for you to tell other people if you want to.

If you don't want to have the drink or take part then you don't have to and you don't need to say why. When you have been taken out of lesson, if you decide you don't want to continue playing the games then you can stop at any time and you don't need to say why.

I hope that you will want to take part and be a scientist helper!

From

Gabbie (the scientist)

Appendix E – Participant assent form.

Participant Assent

I …… am happy to take part in the study 'The effects of fruit drinks on the behaviour and cognition/reading of children' at my school/home.

- ☐ I have been given a piece of paper with information about the study.
- ☐ My parent/guardian has read this to me and explained any parts I didn't understand.
- ☐ My parent/guardian asked if I have any questions about the study and contacted the researcher if I did.
- ☐ I understand that the researchers won't tell anybody else about what I say or do during the study, but I am able to tell other people what I did if I want to.
- ☐ I understand that I don't need to take part if I don't want to.
- ☐ I understand that if I want to stop taking part during the study I can and I don't have to say why.

Name (in capitals) ……

Date…….

Birthday ……(date,month,year)

Appendix F – Low-flavonoid diet sheet.

Low-flavonoid Diet

Please **avoid** eating foods shown below for **24** hours before **each** test session and for the duration of each study day.

- All berries
- Fruit and vegetables (see below for exceptions)
- Fruit juices
- Jams and preserves
- Fruit teas
- Soy products
- Chocolate/cocoa
- Tea (black, green, earl grey etc)
- Coffee
- All high energy and/or caffeinated drinks, e.g. Coca-Cola, Red Bull, Lucozade.
- All dietary supplements

Foods you **may** eat include those shown below.

- Potatoes
- Rice
- Bread
- Sweetcorn
- Mushrooms
- Carrots
- Bananas
- Pasta
- Meat/fish
- Dairy products
- Nuts
- Snacks – crisps, biscuits, cakes (without chocolate or fruit)

Example low-flavonoid diet

Breakfast

Toast with a choice of toppings: Butter/spread, marmite, peanut butter, honey and/or banana.

OR

Croissant(s) with butter (No pain au chocolat).

Snacks (anytime)

Banana, Cantaloupe or Honeydew Melon, Watermelon, Carrots, Brazil nuts, Peanuts, White chocolate, Crisps, Biscuits (no chocolate), Cake (no fruit or chocolate), Pastry-based products (no fruit or chocolate), Cereal bars (no fruit or chocolate), Milk, Cheese, Yoghurt.

Lunch

Sandwich with a choice of fillings: Cheese, ham, other meat, egg, fish, peanut butter, marmite.

OR

Baked white potato with a choice of fillings: Butter/spread, cheese, ham, other meat, egg, fish.

OR

Croissant(s) with butter.

OR

White rice OR pasta with a combination of meat, carrots, sweetcorn, mushrooms, cucumber, iceberg lettuce, parsnips.

Appendix G – York Assessment of Reading for Comprehension (YARC).

G.1 Example YARC passage read aloud to assess reading accuracy, reading rate, and reading comprehension.

In Australia, the reptiles that are known worldwide as monitor lizards, are called goannas. Goannas range in length from 20 centimetres to over 2 metres, but all have the same distinctive shape: a flattened body, long neck with loose skin under the throat, strong legs with long toes and sharp claws, and a long tail.

Mostly they are ground dwellers, hunting close to the burrows in which they live, but they are also good tree climbers and strong swimmers. Being carnivores, they eat lizards, snakes, small mammals, birds, and eggs; often swallowing the animal whole. They hunt their pray by tracking and attacking it, or by using their sharp claws to excavate animals and eggs hidden in the ground.

Like most lizards, goannas lay eggs, usually in a nest or burrow and between seven to thirty-five eggs at a time. These hatch in eight to ten weeks.

Although bulky in appearance, goannas can run swiftly on two legs. They also rear up on two legs when threatened, inflating flaps of skin around their throats, hissing, and lashing out with their powerful tails.

G.2 Example of the YARC scoring tables used to collect raw data.

Form:			Level:			Passage Totals	
Type of reading accuracy error						Reading Accuracy Score	Reading Rate
Mispronunciations	Substitutions	Refusals	Additions	Omissions	Reversals	No. of errors	Time (secs)
Reading comprehension							
Question 1	Question 2	Question 3	Question 4	Question 5	Question 6	Question 7	Question 8

Appendix H – Single Word Reading Test (SWRT) word list.

See	look	play	was	like
This	next	house	going	bell
hang	Stand	their	living	again
First	slowly	score	found	bread
scream	journey	suppose	yawned	should
tissue	caught	stretching	tongue	copies
medicine	strengthen	source	creative	material
eventually	hygiene	despite	calm	journalism
excitable	dehydration	persuade	aggrieved	originate
courageous	atmospheric	familiarise	scenic	recurrence
ferocious	cynical	excursion	coincidental	abysmal
endeavour	rheumatism	haemorrhage	liaise	pseudonym

Appendix I – Rey's Auditory Verbal Learning Task (AVLT) word recall lists.

P	1	2	3	4	5	6
Oil	Egg	Belt	Band	Cage	Gate	Throat
Juice	Ball	Park	Elbow	Sink	Stick	Boot
Jail	Drain	Fox	Flag	Forest	Tent	Rope
Cattle	Palace	Meadow	Letter	Woman	Window	Pedal
Camp	Shed	Brush	Sail	Lake	Toy	Pool
Jewel	Money	Building	Shepherd	Football	Kettle	Tractor
Nail	Coin	Tin	Knee	Stew	Vase	String
Village	Card	Breakfast	Kitten	Candy	Shoulder	Pony
Rock	Toilet	Tool	Bird	Crown	Page	Desk
Berry	wood	Painting	Rubber	Teacher	Island	Ocean
Coat	Bin	Star	Tail	Book	Church	Head
Chest	Paper	Tray	Neck	Hair	Jar	Skirt
Penny	Clock	Garden	Oven	Insect	Picture	Sheet
Leaf	Salt	Meat	Nurse	Soap	Dog	Plate
Gun	Cotton	Spoon	Fruit	Men	Bag	Mud

Appendix J – Test of Word Reading Efficieny-2 (TOWRE-2).

J.1 Example of TOWRE-2 words.

Practise words

on my bee old warm bone most spell

Test words

Form A									
Is	up	cat	red	me	to	no	we	he	the
And	yes	of	him	as	book	was	help	then	time
Wood	let	men	baby	new	stop	work	jump	part	fast
Fine	milk	back	lost	find	paper	open	kind	able	shoes
money	great	father	river	space	short	left	people	waves	almost
Child	strong	crowd	better	inside	plane	pretty	famous	children	without
finally	strange	budget	repress	contain	justice	morning	resolve	describe	garment
business	qualify	potent	collapse	elements	pioneer	remember	dangerous	uniform	necessary

J.2 Example of TOWRE-2 non-words.

Practise non-words

ba um fos gan rup nasp luddy dord

Test non-words

Form A									
Ip	ga	ko	ta	om	ig	ni	pim	wum	lat
Baf	din	nup	fet	bave	pate	herm	dess	chur	knap
Tive	barp	stip	plin	frip	poth	vasp	meest	shlee	guddy
Skree	felly	clirt	sline	dreff	prain	zint	bloot	trisk	kelm
Lunaf	cruddy	trober	depate	glant	splosh	dreker	ritlun	hedfert	bremick
nifpate	brinbert	clabom	drepnort	shrattec	plofent	smucrit	pelnador	fornalask	fermabalt

Appendix K – Positive and Negative Affect Scale for Children (PANAS-C).

This scale consists of a number of words that describe different feelings and emotions. Read each item and then circle the appropriate answer next to that word. Indicate to what extent you are feeling that emotion right now.

1 = Very slightly 2 = A little 3 = Moderately 4 = Quite a bit 5 = Extremely

Interested	1	2	3	4	5
Sad	1	2	3	4	5
Frightened	1	2	3	4	5
Alert	1	2	3	4	5
Excited	1	2	3	4	5
Ashamed	1	2	3	4	5
Upset	1	2	3	4	5
Happy	1	2	3	4	5
Strong	1	2	3	4	5
Nervous	1	2	3	4	5
Guilty	1	2	3	4	5
Energetic	1	2	3	4	5
Scared	1	2	3	4	5
Calm	1	2	3	4	5
Miserable	1	2	3	4	5
Jittery	1	2	3	4	5
Cheerful	1	2	3	4	5
Active	1	2	3	4	5
Proud	1	2	3	4	5
Afraid	1	2	3	4	5
Joyful	1	2	3	4	5
Lonely	1	2	3	4	5
Mad	1	2	3	4	5
Fearless	1	2	3	4	5
Disgusted	1	2	3	4	5
Blue	1	2	3	4	5
Daring	1	2	3	4	5
Gloomy	1	2	3	4	5
Lively	1	2	3	4	5

Appendix L – 3-day food diary.

	Time	<day>	<day>	<day>
Breakfast				
Snack				
Lunch				
Snack				
Dinner				
Snack				
Water □ = 1 x 250ml cup		□□□□□□□	□□□□□□□□	□□□□□□□□

Appendix M – Chronic treatment administration details.

Title of study: Effects of chronic wild blueberry supplementation on cognition, reading behaviour, and mood, in seven to ten year olds.

Schedule of drink consumption

Your child must consume 1 drink every day for 4 weeks. Your child must finish all contents.

If something goes wrong…

If you spill the formula or drink – If you spill over ¼ of the frozen formula, or the drink after it has been prepared, then simply make up another drink using a fresh bag of frozen formula, water and orange squash and discard the previously spilt waste.

If your child does not drink the drink - In the event that your child refuses to drink the drink one day, you must try to administer another (fresh) drink that day. If your child still does not comply, you should not administer the drink that day and you should record it for the researcher.

If your child is sick and/or vomits –If your child is feeling sick or vomits before the drink's consumption, please refer to 'If your child does not drink the drink'. If your child vomits after the drink's consumption please record this for the researcher and do not administer another drink that day. Continue drink consumption as normal the following day.

If I am getting low on frozen formula – You will have been given 2 weeks' worth of the frozen formula and 1 bottle of Rock's orange squash to use to make up a drink each day. If you are getting low on frozen formula before the next test session and do not think you will have enough until then, please contact the researcher via email. They will then arrange a convenient time to either drop more formula round to your home, meet you at your child's school, or for collection in the Psychology Department. You should not get low on Rock's Orange Squash due to you only needing 30 ml per day. You should not use the orange squash for any other reason than to make your child's drink each day (i.e. do not let your child have it throughout the day or give to other children in the household).

If your child has any unexplainable symptoms – In the unlikely event that your child starts developing symptoms such as a rash or allergic reaction during the first week of the study, and you think this is due to the fruit drinks, then stop administration and contact the

researcher. The fruit juice formulas are 100% safe for consumption and comply with all food safety standards. Ingredients are natural and are present in common foods we eat. The researcher will have already asked you before the start of the study whether your child has a fruit or fruit juice intolerance. If so, we would not have allowed your child to participate. If you think circumstances may have changed then we advise you to contact your GP for a test.

How to make the fruit drinks…..

1. Measure 170 ml cold water and pour into the flask provided.
2. Measure 30 ml Rock's orange squash and pour into the flask.
3. Remove a sachet from the freezer and squeeze to remove any lumps.
4. Tear the seal of the sachet and carefully pour the contents into flask.
5. Fix the lid onto the flask and shake well for 1 minute.
6. Ensure your child consumes the drink within 30 minutes.

Appendix N: Treatment administration log.

Day	Consumed	Time consumed	Not consumed	Comments

Appendix O: Orthographic stories.

Practise story: George went to the farm, and saw lots of animals. George passed by the sheep [practise word]. Sheep are his favourite animal. George wanted to feed the sheep. When the farmer arrived, George was allowed to feed the sheep

Test stories

Story 1: Ben was at the pet shop and the fish tank looked dirty. Ben picked up the [non-word]. The [non-word] is used to clean fish tanks. Ben placed the [non-word] in the fish tank. When the fish tank was clean, Ben put away the [non-word].

Story 2: Pip went to the beach today, so he got sand all over his body. Pip passed by the [non-word]. The [non-word] is used to remove sand from the body. Pip put his body near the [non-word]. When all the sand was removed from his body, Pip left the [non-word].

Story 3: Kim wanted to sit by the window, but it was at the top of the wall. Kim put on the [non-word]. The [non-word] is used to climb walls. Kim pressed the [non-word] against the wall. After she reached the top of the wall, Kim took off the [non-word].

Story 4: Pam went to the garden, and the flowers looked dull. Pam took out of the [non-word]. The [non-word] is used to make flowers look bright. Pam moved the [non-word] around the flowers. When the flowers looked bright, Pam put back the [non-word].

Story 5: Sara went outside during the storm, so her hat got wet. Sara ran to the [non-word]. The [non-word] is used to dry hats. Sara put her hat on the [non-word]. Once her hat was dry, Sara walked away from the [non-word].

Story 6: Nick received a new game, but the cards were not shuffled. Nick took out the [non-word]. The [non-word] is used to shuffle cards. Nick put the cards into the [non-word]. When the cards were shuffled, Nick put back the [non-word].

Story 7: Sam went into the forest, and the birds were quiet. Sam turned on the [non-word]. The [non-word] is used to make the birds sing. Sam placed the [non-word] near the birds. Once the birds began to sing, Sam turned off the [non-word].

Story 8: Max was about to eat, but he did not want peas in his meal. Max started the [non-word]. The [non-word] is used to remove peas from meals. Max put his meal into the [non-word]. When all the peas were removed from his meal, Max stopped the [non-word].

Appendix P: Reanalysis of the episodic memory and reading behaviour data from Study 1.

For this reanalysis of the episodic memory and reading behaviour data, Baseline was removed as a covariate and replaced as a fixed effect to examine the direct effects of baseline performance on later performance at two hours, and any subsequent interactions with treatment (see Section 2.3 for the original statistical analysis for Study 1).

N1: AVLT

Baseline performance significantly predicted later performance at two hours for following AVLT outcome measures, regardless of treatment group (Table N1): word span; $F(6,51) = 3.02$, MSE = .99, $p = .013$, $n^2 = .21$, words learnt; $F(11,51) = 3$, MSE = 2.20, $p = .004$, $n^2 = .19$, final acquisition; $F(11,51) = 5.67$, MSE = 1.1, $p < .001$, $n^2 = .35$, retroactive interference; $F(11,51) = 2.51$, MSE = 2, $p = .013$, $n^2 = .05$, short delayed recall; $F(10,51) = 4.28$, MSE = 2.88, $p < .001$, $n^2 = .53$, long delayed recall; $F(9,51) = 2.85$, MSE = 3.79, $p = .008$, $n^2 = .31$, total delayed recall; $F(19,51) = 4.95$, MSE = -1.5, $p < .001$, $n^2 = .41$, total word recall (A1-A5); $F(29,51) = 6.54$, MSE = -23, $p < .001$, $n^2 = .48$, and word recognition; $F(11,51) = 5.76$, MSE = 2.07, $p < .001$, $n^2 = .26$. For all measures, the means (Table 2.4.2.1A) indicated that those who had greater word recall performance at baseline had greater performance two hours later, regardless of treatment. Baseline performance did not predict later performance for proactive interference.

There was a significant Treatment x Baseline interaction for final acquisition; $F(7,51) = 2.94$, MSE = 1.84, $p = .012$, with pairwise comparisons revealing that those who had greater performance at baseline (7 words recalled or more) had greater performance two hours after WBB intake compared to placebo. The significant Treatment x Baseline interaction for total delayed word recall; $F(7,51) = 3.18$, MSE = 4.29, $p = .007$, $n^2 = .06$, with pairwise comparisons revealing that hose who had greater performance at baseline (13 words recalled or more) had greater performance two hours after WBB intake compared to placebo. There was a significant Treatment x Baseline interaction for total word recall; $F(7,51) = 2.18$, MSE = 6.59, $p = .051$, $n^2 = .04$, with pairwise comparisons revealing that those who had greater performance at baseline (28 words recalled or more) had greater performance two hours after WBB intake compared to placebo. Last, the significant Treatment x Baseline interaction for word recognition; $F(9,51) = 2.22$, MSE = 2.46, $p = .035$, $n^2 = .04$, with pairwise comparisons revealing that those who had greater performance at

baseline (7 word recalled or more) had greater performance two hours after WBB intake compared to placebo.

Further, the fixed effect of Treatment significantly predicted retroactive interference; $F(1,51) = 4.2$, MSE = .96, $p = .046$, $n^2 = .08$, however pairwise comparisons found no difference in interference levels between treatment groups. The fixed effect of Treatment also significantly predicted total word recall; $F(1,51) = 9.79$, MSE = 4.66, $p < .005$, $n^2 = .16$, however pairwise comparisons revealed no difference in performance between treatment groups. The fixed effect of Treatment was a near significant predictor of final acquisition; $F(1,51) = 3.49$, MSE = 1.1, $p = .067$, $n^2 = .06$, however pairwise comparisons found no difference in performance between treatment groups.

Table N1: Adjusted β coefficients and *p* values for the effect of treatment on all AVLT LMM analyses at two hours when Baseline was included as a fixed effect.

	Fixed effects	**β coefficients**	***p* values**
Word Span			
	Treatment	< .001	.547
	Baseline	-1.5	.013
	Treatment*Baseline	-1.5	.494
Words Learnt			
	Treatment	1.17	.850
	Baseline	-2.17	.004
	Treatment*Baseline	-.17	.463
Final Acquisition			
	Treatment	.50	.067
	Baseline	-3	< .001
	Treatment*Baseline	-1.5	.012
Proactive Interference			
	Treatment	-2.2	.814
	Baseline	-1	.153
	Treatment*Baseline	3.7	.208

Table N1: *continued…*

Fixed effects		**β coefficient**	***p* value**
Retroactive Interference			
	Treatment	-1.15	.046
	Baseline	-3.15	.013
	Treatment*Baseline	-1.85	.742
Short Delayed Recall			
	Treatment	3.33	.641
	Baseline	-1.67	< .001
	Treatment*Baseline	-2.83	.421
Long Delayed Recall			
	Treatment	< .001	.560
	Baseline	-3	.008
	Treatment*Baseline	-1.5	.443
Total Delayed Recall			
	Treatment	7	.423
	Baseline	-1.5	< .001
	Treatment*Baseline	-9	.007
Total Recall (A1-A5)			
	Treatment	-11	.003
	Baseline	-23	< .001
	Treatment*Baseline	11	.051
Word Recognition			
	Treatment	< .001	.112
	Baseline	< .001	< .001
	Treatment*Baseline	3	.03

N2: TOWRE

First, baseline performance significantly predicted SWE performance at two hours, regardless of treatment group; $F(35,51) = .402$, $MSE = 2.25$, $p < .001$, $n^2 = .85$, with means (Table 2.4.2.4A) indicating that those who had greater performance at baseline had greater performance at two hours. Baseline performance also significantly predicted PDE

performance at two hours, regardless of treatment group; F(31,51) = 48.01, MSE = .2.88, p < .001, n^2 = .85, with means (Table 2.4.2.4A) indicating that those who had greater performance at baseline had greater performance at two hours.

There was a significant Treatment x Baseline interaction for SWE; F(6,51) = 15.18, MSE = 2.98, p < .001, n^2 = .45, with pairwise comparisons revealing that those who had poorer performance at baseline (41-51 words) had poorer performance at two hours after WBB intake compared to placebo. Further, those who had greater performance at baseline (68 words or more) had greater performance at two hours after placebo compared to WBB.

There was a significant Treatment x Baseline interaction for PDE; F(6,51) = 2.35, MSE = 3.81, p = .044, n^2 = .04, with pairwise comparisons revealing that those who had greater performance at baseline (29 non-words or more) had greater performance two hours after WBB intake compared to placebo. Further, those who had poorer recall had baseline (12 non-words or less) had poorer performance two hours after placebo intake compared to WBB.

The fixed effect of Treatment did not predict SWE or PDE (Table N2).

Table N2: Adjusted β coefficients and *p* values for both SWE and PDE LMM analyses at two hours when Baseline was included as a fixed effect.

	Fixed effects	**β coefficient**	***p* value**
SWE			
	Treatment	-1	.529
	Baseline	-55	< .001
	Treatment*Baseline	-7	< .001
PDE			
	Treatment	-1.5	.143
	Baseline	-45	< .001
	Treatment*Baseline	.7.5	.044