# The impact of Ārepa, a blackcurrant-based nootropic drink, on neurocognitive function

*A secondary analysis of the LINK study*

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# Abstract

**Background:** Brain health is essential for overall well-being, but is a growing concern in society today. A variety of dietary factors have been shown to support brain health, with polyphenols in particular showing significant potential cognitive function. Compared to other polyphenol-rich fruits, blackcurrants remain largely unexplored for their potential benefits for cognitive health.

**Methods:** Forty healthy females participated in this double-blinded, randomised, placebo-controlled cross-over trial. Participants were recruited with a balance of 'optimal' and 'sub-optimal' diets according to a dietary screening tool. The active intervention was the Ārepa performance beverage (300mL), a polyphenol-rich blackcurrant drink (311mg anthocyanins) also containing l-theanine (200mg) and pine-bark extract (150mg), which was compared to a taste- and colour-matched placebo beverage. The trial was three months in duration, including four weeks on each intervention arm and a four-week wash-out period between. Cognition was assessed using the Purple-Multi-Tasking Framework (MTF), comprising of four concurrent tasks including mental arithmetic, letter recall, visual tracking, and Stroop tests. Stress reactivity was assessed using the State-Trait Anxiety Inventory: State Subscale and Visual Analogue Mood Scales before and after completing the 20-minute MTF, which acts as a cognitive stressor. Differences in changes in cognitive performance and stress reactivity from baseline to follow-up between intervention groups were assessed by a series of paired t-tests.

**Results:** There was a trend towards improvements in the MTF total (+1689) and letter search scores (+955) following four weeks of daily Ārepa supplementation ($p= 0.052$ and 0.057, respectively), indicating improvements in working memory and executive function. When investigating the effect of baseline diet quality on intervention responses, we found that these benefits to MTF total and letter search scores were driven by participants in the 'sub-optimal' diet group ($p= 0.028$ and 0.022, respectively), while no benefit was observed in the 'optimal' diet group ($p= 0.774$ and 0.921, respectively). No difference in change for stress reactivity measures (alertness, calmness, contentment, fatigue, and stress) was seen between active and placebo beverages.

**Conclusion:** These findings add to a body of evidence demonstrating the benefit of polyphenols in supporting specific domains of cognition, including executive function and working memory. Our findings do not support the role of polyphenols in improving stress reactivity measures in response to a cognitive stressor. These findings warrant future research to examine mechanistic actions, informing recommendations for the promotion of cognitive health, and the potential attenuation of cognitive decline.

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Ngā mihi maioha.

# Statement of Contribution

| Researcher | Contribution to thesis |
|---|---|
| Jessica Miller<br>*MHSc student* | • Primary author of this thesis<br>• Data cleaning, processing, and scoring in Excel<br>• Analysis of food records in FoodWorks<br>• Data analysis in SPSS<br>• Interpreted results, and integrated findings with previously published research |
| Dr Nicola Gillies<br>*Academic supervisor* | • Application for ethical approval<br>• Developed study design<br>• Recruitment of study participants<br>• Conducted randomised control trial<br>• Reviewed and edited thesis chapters |
| Chris Frampton<br>*Statistician* | • Consultation on statistical analysis methodology<br>• Guidance on using SPSS |
| Ella Risbrook<br>*MHSc student* | • Cross-checked food records |

# Table of Contents

# List of Tables

# List of Figures

# Glossary

| | |
|---|---|
| AGEs | Advanced glycation end products |
| AI | Adequate Intake |
| Ārepa | Commercially available blackcurrant nootropic drink, containing 1297mg polyphenols (of which 311mg anthocyanins), 200mg L-theanine, and 150mg Enzogenol® |
| Aβ | Amyloid-beta |
| BBB | Blood-brain barrier |
| BDNF | Brain-derived neurotrophic factor |
| BMI | Body Mass Index |
| CANTAB | Cambridge Neuropsychological Test Automated Battery |
| CNS | Central nervous system |
| DST | Dietary Screening Tool |
| EAR | Estimated Average Requirements |
| Enzogenol® | New Zealand pine bark extract |
| L-theanine | Unique non-protein amino acid |
| LINK study | Polyphenol-rich drink for gut and brain health |
| MTF | Multi-Tasking Framework |
| RCT | Randomised control trial |
| ROS | Reactive oxygen species |
| SMILES | Supporting the Modification of lifestyle in Lowered Emotional States |
| SPSS | Statistical Package for the Social Sciences |
| STAI-S | State-Trait Anxiety Inventory- State Subscale |
| VAMS | Visual Analogue Mood Scales |

# Chapter 1: Literature Review

## 1.1 Introduction

The brain is a complex organ in the human body, regulating processes in both the central and peripheral nervous systems (1). Rather than a fixed, unchangeable organ as once thought, we now know that the brain is a dynamic, malleable organ which continually experiences functional and structural changes in response to environmental, physiological and lifestyle factors (2,3). This has led to growing interest in the role of lifestyle factors such as diet on brain health (4), which is essential for overall well-being (5). Brain health refers to the state of brain function across cognitive, social-emotional, sensory, behavioural, and motor domains, allowing an individual to attain their full potential throughout the life course, irrespective of the presence or absence of illness (1).

Cognitive health more specifically refers to the mental processes that enable perception, thought, comprehension, communication, and executive function. This has been a topic of growing interest among the general population and research communities, while the prevalence of neurocognitive impairments are increasing in parallel, with cases estimated to have doubled over the last decade (6). As the global population ages, the burden of neurological disorders and challenges to preserve cognitive health continue to rise (7). Posing a significant societal and economic burden globally, dementia alone is estimated to affect 55.2 million and costing US $1313 billion annually (8). Cognitive impairment has been identified in a variety of neurological and neuropsychiatric conditions, including dementia, depression and anxiety (9). Cognitive health is important to perform both the basic and complex mental demands of daily living. Across the general work environment, there is an ongoing shift from physical, manual labour occupations to those characterised by increasing mental demands (10). Maintaining healthy cognitive function across the life course is therefore important in optimising health and longevity (7).

In addition to cognitive health, mental health is another important aspect of brain health. We now understand that cognitive health and mental health are inherently connected, with cognitive ability predicting levels of psychological well-being and incidence of psychiatric disorders (9). Referring to psychological and emotional well-being, mental health encompasses the mind, thoughts, feelings, and behaviour. Psychological stress reactivity is an interesting but underappreciated concept linked to mental health, and is defined as an individual's capacity to respond to stressors. Dysregulated stress

reactivity has been identified as a potential mechanism by which psychological stress contributes to the development of mental health and disease outcomes, including depression, anxiety, and poorer cognitive ability (11). Among the major causes of disability globally, mental ill-health is an increasing public health concern, with an estimated one billion, or one-in-eight people worldwide living with mental illness (12,13). However, society and health systems have historically neglected and under-resourced mental health (14). As the collective burden of poor brain health is substantial, this topic warrants greater consideration and exploration.

## 1.2 Diet and brain health

The continual rise in the prevalence of neurological and neuropsychiatric conditions necessitates the development of broad strategies that can promote good cognitive and mental health across the life course (15). Despite extensive research conducted on brain diseases, only recently has considerable effort been dedicated to exploring the impact of lifestyle-related factors on brain health (15). The role of diet in cognitive and mental health has been a topic of growing interest among research communities (16), and foods or nutrients that have recognised in the past solely for their positive effects on physical health have now been discovered to confer benefits for brain health (15). For example, certain dietary patterns and bioactive compounds have been shown to support cognitive function and protect against cognitive decline (4). Emerging research has identified that dietary factors can affect the development and preservation of neurons, as well as the protection of the brain from insult linked with neurological illnesses and injuries (15). Similarly, observational evidence across numerous countries and age groups supports the hypothesis that diet quality is a potential protective factor and risk factor for mental health and mental ill-health, respectively (17–20). The strongest available evidence points towards a protective effect of a Mediterranean-style diet (rich in in fruit, vegetables, whole grains, legumes, and fish) for both cognitive function and mental health, while the inverse is true for processed foods (including refined grains, sugary products and fried foods) (4,21–23).

Cognisant of the limitations of observational research, the reverse causality hypothesis which posits that poor mental health or cognitive function leads to poor dietary behaviours is entirely plausible. However, there is a growing evidence base of intervention studies which highlight that dietary modification may produce an effective and accessible treatment option for the enhancing cognitive function and mental well-being (21,24). Notably, these effects are seen across a range of

experimental paradigms, from acute to longer-term interventions and from individual nutrients to whole of diet approaches (23,25). For example, consumption of the flavanol phytonutrient derived from cocoa has resulted in acute improvements in both mood and cognitive performance, within the space of 90 minutes (26). A Mediterranean diet has shown to support short-term cognition and mood within ten days, with significant improvements in alertness, attention, and contentment (25). A simple increase in fruit and vegetable consumption for two weeks led to improved psychological well-being among young adults, including increases in self-reported vitality, flourishing, and motivation (24). Looking to longer-term interventions, 12-weeks of a modified Mediterranean diet resulted in significant improvement in depressive symptoms among individuals with major depressive disorder in the landmark SMILES trial (21). Within the broad scope of how diet can be modified to improve brain health presented above, polyphenols have garnered particular and consistent attention for their potential benefits for brain health (27).

## 1.3 Polyphenols

Phytonutrients are naturally occurring chemicals produced by plants as defence mechanisms against physical damage, pathogen insults, and ultraviolet radiation (28). In the last decade, evidence has emerged regarding the health benefits of a class of phytonutrients known as polyphenols. Research has identified that dietary patterns rich in polyphenols have various benefits to health, including anti-inflammatory, anticarcinogenic, cardioprotective and neuroprotective effects (28).

Polyphenols are bioactive compounds characterised by their phenolic structure present in plant-based foods such as fruit, vegetables, teas, and spices (Table 1.1). Foods and beverages rich in dietary polyphenols include berry fruits, cherries, apples, pomegranate, citrus, grapes, cacao, wine, coffee, and tea (29–31). In plant-based foods, dietary polyphenols contribute to their bitterness, flavour, astringency, colour, aroma, and oxidative stability (28). With over 8000 different polyphenolic compounds identified, these polyphenols are classified into four main classes: phenolic acids, flavonoids, stilbenes, and lignans (28). Flavonoids have a distinctive 2-phenyl-1,4-benzopyrone chemical structure, comprise the most abundant polyphenolic group, and include six subgroups: anthocyanins, flavanones, flavanols, flavones, flavonols, and isoflavanoids (32).

Table 1.1. Common polyphenolic compounds and their dietary sources

| Polyphenol class | Common compounds | Dietary sources[1] |
|---|---|---|
| **Flavonoids** | Anthocyanins | Blackcurrant, berries, cherry, plum, pomegranate, red cabbage |
| | Isoflavones | Soybean, soy products |
| | Flavanols | Apple, cocoa, grapes, red wine, tea |
| | Flavanones | Citrus |
| | Flavonols | Apple, broccoli, onion, tea |
| | Flavones | Capsicum, celery, lettuce, onion |
| **Phenolic acids** | Hydroxybenzoic acid | Blueberry, cranberry, strawberry, tea |
| | Hydroxycinnamic acid | Apple, cocoa, coffee, eggplant, pear |
| **Lignans** | Secoisolariciresinol | Flaxseed |
| **Stilbenes** | Pterostilbene | Berries, grapes |
| | Resveratrol | Grapes, red wine |

[1]Food and beverages rich in polyphenols (33–35).

Anthocyanins are responsible for contributing to the red, purple, and blue pigments of a variety of fruits and vegetables, and are the most commonly consumed polyphenolic compound in diets today. This group of polyphenols have gained significant attention as possible treatment options for neurodegenerative diseases (36), having demonstrated their potential as neuroprotective agents, both in vitro and in vivo (37,38).

## 1.4 Anthocyanins and brain health

### 1.4.1 Mechanistic insight gained from in vitro experimentation

A variety of studies performed in vitro have demonstrated the effect of polyphenols on the brain, including neuronal and synaptic processes. In vitro, crude fruit extracts, isolated or combined anthocyanins, as well as anthocyanin metabolites have been analysed within primary cultures of neurons and neuronal cells to explore the neuroprotective potential of anthocyanins (39–42). Anthocyanins were shown to benefit the central nervous system (CNS) through modulation of neuroinflammation, apoptosis, oxidative stress, induced neurotoxicity, and toxic actions pertaining to excitatory neurotransmitters (40,42).

*Blood-brain barrier*

Before describing mechanistic actions in more detail, the accessibility of polyphenols in the CNS is a vital aspect of directly supporting neuroprotective activity which needs mention (38). To reach the target brain tissues, polyphenols must transport across a significant obstacle, the blood-brain barrier (BBB), at pharmacologically effective concentrations (43). As a crucial feature of the CNS, the BBB has a protective role by preventing substances into the brain, and providing essential nutrients, hormones, and drugs to brain tissues (44). One *in vitro* study explored the transport of three different flavonoids (anthocyanins, flavonols, and flavan-3-ols), and their metabolites across a human blood-brain model (45). All three flavonoids and their metabolites crossed the BBB in a time-dependent manner, though the phenolic metabolites typically demonstrated greater transport efficiency than their parent compounds (45). The ability of anthocyanins to cross the BBB has been attributed to their lipophilic character, membrane transporters, and unique molecular structure (43,46).

*Antioxidant effects*

An *in vitro* study investigated anthocyanins' neuroprotective effects on neuroblastoma cells treated with anthocyanin-rich fruit juices (blueberry and cranberry) and cyanidin (29). The cranberry juice, blueberry juice, and particularly cyanidin decreased unfavourable intracellular reactive oxygen species (ROS) production and lipid peroxidation following hydrogen peroxide-induced neurotoxicity by directly

scavenging free radicals (29). The activity of antioxidant enzymes catalase and superoxide dismutase were also upregulated by cyanidin and anthocyanin-rich fruit juices (29). In a separate study, anthocyanins decreased ROS generation and induced antioxidant gene expression following $A\beta_{1-42}$-induced neurotoxicity in neuroblastoma cells (42). Anthocyanins have also demonstrated anti-inflammatory potential by reducing pro-inflammatory mediators and counteracting oxidative stress in $A\beta_{1-42}$-treated BV2 microglia cells and neuroblastoma cells through antioxidant activities (42,47).

These antioxidant effects may also extend towards protecting against neurocognitive diseases, such as Alzheimer's disease. Amyloid-beta (Aβ) peptides are frequently utilised *in vivo* as detrimental oxidative agents to assess neuronal viability in Alzheimer's disease (42,48). Pathophysiological indicators thought to be linked with neuroinflammation in Alzheimer's disease are neurofibrillary tangles and neuritic plaques, including $A\beta_{1-42}$ plaques (49). These features produce inflammatory, oxidative, and pro-apoptotic actions (50). One study found anthocyanins lowered Aβ levels in $A\beta_{1-42}$-injected neuroblastoma SH-SY5Y cells by reducing amyloid precursor protein and b-secretase 1 expression, key factors contributing to the formation of Aβ aggregates (42). Additionally, the metabolite of cyanidin-3-glucoside, known as protocatechuic acid, has shown to destabilise Aβ fibrils in PC12 (rat pheochromocytoma) cells (51). The attenuation of Alzheimer's markers, neuro-inflammatory markers, and neuro-apoptotic markers by anthocyanins *in vitro* demonstrates their complexities in protecting neuronal cells against Aβ toxicity (42).

*Neuroprotection*

Evidence has also demonstrated the neuroprotective potential of anthocyanins through anti-glycation effects (39). Glycation is a process initiated by a chemical reaction between reduced sugars and proteins to produce unstable aldimine and ketoamine structures (39). Gradually, these structures transform into a diverse group of compounds collectively known as advanced glycation end products (AGEs). Recent studies have hypothesised that AGEs may contribute to the pathogenesis of cognitive decline and various neurogenerative diseases such as Alzheimer's disease (52–54) An in vitro study analysed anthocyanin-rich berry extracts (100μ/mL), revealing their ability to trap reactive carbonyl species, scavenge free radicals, and inhibit glycation compared to anthocyanin-free extracts (39). Another in vitro study revealed the anthocyanin cyanidin-3-glucoside demonstrated neuroprotective capabilities through inhibiting neuronal necrotic-like cell death by 37% (40). These results demonstrate the neuroprotective potential of common anthocyanin-rich berries.

The ability of anthocyanins to support cognitive health and treat neurodegenerative diseases may be restricted, due to its relatively low bioavailability in the brain following ingestion (41). Collectively, in vitro work has paved the mechanistic plausibility of anthocyanins to promote brain health, including the capacity to cross the BBB, antioxidant and anti-inflammatory activities, and neuroprotective potential. However, a pivotal issue here is the fact of low bioavailability. Anthocyanin's favourable properties may be limited by their in vitro concentrations, because elevated levels of this polyphenol can become harmful to cells (38). It is also important to highlight that physiological concentrations and actions of polyphenols using biophysical and in vivo assays may not translate to those completed in vitro (45,55). Further studies utilising in vivo models are imperative to consider when evaluating the potential actions of polyphenols on the brain, in both health and disease (45,55).

### 1.4.2 Mechanistic insight gained from animal studies and human clinical trials

*In vivo* animal studies and human clinical trials have confirmed some of the mechanisms proposed by in vitro studies described above, such as modulation of neurodegeneration, neuroinflammation, and oxidative stress (38). The supplementation of polyphenols within *in vivo* animal studies and human clinical trials have further added to the potential mechanistic actions, including regulation of synaptic plasticity, removal of cellular toxical proteins, and improvement in cerebrovascular blood flow (56–59). Literature has also revealed the mechanistic actions by which anthocyanins influence cognitive function appear to differ with duration of exposure (38). Acute anthocyanin supplementation is likely associated with improved cerebrovascular blood flow, whereas long-term anthocyanin supplementation may promote morphological changes (38).

The proposed mechanisms appear to relate to benefits to different cognitive domains. For example, anthocyanins seem to influence memory through their ability to interact with physiological and molecular components involved in memory processing (60). In the brain, anthocyanins and their metabolites modulate neuronal signalling pathways involved in synaptic plasticity, which are linked to memory acquisition and consolidation (61). Anthocyanins may also support memory formation through the modulation of brain-derived neurotrophic factor protein (BDNF) gene transcription. BDNF is important for neuronal growth, neuronal differentiation, and synaptic plasticity, all of which contribute to cognitive function, including learning and memory. *In vivo* animal studies have demonstrated that polyphenol supplementation has increased circulating BDNF (62–65). Polyphenol supplementation has also shown to significantly enhance acute cerebrovascular blood flow in human

clinical trials (58,59). Increased cerebrovascular function facilitates neurogenesis in the hippocampus, with new cells clustering near blood vessels, potentially supporting memory function (61). Efficient cerebrovascular function is critical for optimal brain function, with research indicating decreased cerebral blood flow in dementia patients (66). The exact mechanisms underlying the neurocognitive benefit of polyphenols in humans remains complex and inconclusive, requiring further research to strengthen the evidence base (67).

## 1.4.3 Clinical evidence

### 1.4.3.1 Animal studies

In the late 1990s, preliminary evidence of the potential benefit of polyphenol intake on cognition emerged, showing that strawberry and blueberry extracts successfully counteract age-related deficits in neuronal and behavioural measures of rodents (68,69). The ability of anthocyanins to cross the BBB, as well as their presence in regions of the brain, including the hippocampus, cortex, cerebellum, and striatum of rats prompted researchers to further explore the benefits and mechanisms of these compounds in neuropsychology (70,71). Currently, in vivo research on the potential neuroprotection of polyphenols in animal models are providing valuable information regarding direct and indirect actions of these compounds to combat age-related neurological decline and pathological neurodegeneration (63,72,73).

*In vivo* animal studies have explored the effect of polyphenols, particularly chronic anthocyanin supplementation on a range of neurocognitive outcomes (65,74–78). Animal studies involving long-term anthocyanin supplementation have demonstrated positive effects on cognition, including improved memory function and spatial learning (65,74–78). Despite differing considerably in methodology, including anthocyanin type, anthocyanin dose, and study duration, the animal studies were consistently found to enhance memory function (65,74–78). For example, a trial involving aged rats fed a polyphenol-supplemented diet of either pure anthocyanins or pure flavanols for six weeks demonstrated significant improvement in spatial working memory ($p=0.006$ and $p=0.002$ respectively) (65). Another study involving four-week administration of anthocyanin-rich *Vaccinium ashei* berry extract significantly improved long-term memory retention among mice (78). There is also a body of evidence exploring the effect of a variety of anthocyanin-rich fruits on memory performance among aged rodents (74–77). Various rodent studies, including those involving long-term anthocyanin-rich

cherry, blackberry, and blueberry supplementation, consistently reported improvement in spatial working memory and some demonstrating attenuation in age-related cognitive decline (74–77).

*In vivo* animal studies exploring the effect of long-term anthocyanin supplementation on other cognitive domains such as motor performance among aged rodents have yielded more conflicting results (74,75). For instance, positive outcomes were observed in an eight-week study whereby a 2% blackberry-supplemented diet significantly improved coordination and balance in rats, while a similar study with blueberry- or strawberry-supplemented diets also demonstrated enhanced motor skills in aged rats (69,74). Conversely, other studies have disputed the association between anthocyanin supplementation and motor performance among aged rodents (75,79,80). For example, studies involving anthocyanin-rich tart cherry supplementation, strawberry, and Concord grape juice found no significant effects on motor performance among rodents (75,79,80). The discrepancies in findings have been attributed to inadequate anthocyanin dosages, or extent of age-related motor deficits at the time of intervention (75).

In vivo animal studies investigating long-term anthocyanin supplementation on neurocognitive outcomes consistently demonstrate positive effects on cognitive performance, particularly memory function (65,74–77). It is important to note that neurocognitive improvements were often coupled with physiological changes, proposing potential mechanisms responsible for enhancing cognition. Examples of physiological impacts included improvements in hippocampal autophagy, hippocampal angiogenesis, hippocampal brain-derived neurotropic factor, and neuronal spine density, as well as protection against radical-induced deoxyribonucleic acid damage in the brain (65,78,81).

There is increasing literature researching transgenic models and anthocyanin treatments supporting the prevention of cognitive decline, reduction of neurodegeneration, and improvement of memory formation (72,73,82–84). The majority of studies are consistent and guided by distinct mechanistic actions, demonstrating the potential of anthocyanins in counteracting age-related neurological and cognitive decline (38). However, translating the dose and frequency of polyphenolic interventions of animal models to human trials has remained a challenge to be addressed by research (38). There is a lack of consensus from research regarding the minimum dose and frequency of polyphenol consumption to attain sufficient effects in combating neurological disorders within animal studies (38). It is also important to note, the concentrations of polyphenols administered to animals are considerably higher than those present in dietary intakes in clinical human trials (38).

### 1.4.3.2 Observational evidence

Before discussing clinical trials, it is worth noting the robust body of observational evidence adds support to the relationship between dietary flavonoid intake and cognitive health (85,86). A cross-sectional study found participants habitually consuming flavonoid-rich foods, including chocolate, tea, and wine had superior cognitive test scores and lower risk of poor cognitive performance (85). Notably, the association between flavonoid-rich foods and cognitive function was dose-dependent, with maximum effect at daily intakes of 10g and 75-100mL for chocolate and wine, respectively (85). Similarly, a prospective cohort study found a higher intake of total flavonoids was associated with reduced risk of subjective cognitive decline, following adjustment for co-founding variables (86). A dose-response model was observed, with the steepest curve for flavones, followed by anthocyanins. In a recent longitudinal study, highest compared with the lowest dietary intake of anthocyanins was associated with lower risk of developing Alzheimer's disease and related dementia over a 19.7 year follow-up period (87). Overall, observational evidence has identified a dietary pattern containing flavonoid-rich foods is associated with maintenance of cognitive function, in a dose-dependent manner (85). However, as observational evidence has limitations, including the ability to demonstrate causality, it is also important to consider human clinical trials.

### 1.4.3.3 Human clinical trials

Recent human studies have identified polyphenols, particularly anthocyanins as key compounds in supporting cognition, through supressing neuroinflammation, enhancing vascular function, and deaccelerating age-related neurodegeneration (88–91). Both acute and long-term consumption of anthocyanin-rich fruits have demonstrated potential in supporting cognition in clinical trials involving young and older adults, with or without some degree of cognitive impairment (88,89,92). The acute and long-term anthocyanin supplementation studies are summarised in Table 1.2 and Table 1.3, respectively. Note- both tables are an overview relevant for the purpose of this thesis, for an extensive review of the findings refer to the meta-analysis by Lamport and Williams (67).

*Acute anthocyanin supplementation*

Acute supplementation studies have explored the neurocognitive effect of a variety of anthocyanin-rich fruits (Table 1.2). Blueberries have been most extensively studied in acute human clinical trials (93–98), and particularly so among school-aged children (7-10 years) (93–97). These randomised control trials (RCTs) were mostly double-blind, placebo-controlled and cross-over by design, and involved consumption of a blueberry treatment beverage, containing anthocyanin doses between 127-253mg. Containing significant heterogeneity in cognitive testing, the studies contained a variety of neurocognitive tools and assessed cognition between 1.15-6 hours post-consumption of blueberry product. Although these studies produced mixed results, a single dose of anthocyanin-rich blueberry beverage trended towards supporting cognition, especially memory and attention function. A notable study involving blueberry beverages with two different anthocyanin doses (127mg and 253mg) demonstrated a dose-response model, with the worst cognitive performance followed the placebo and the best following the higher anthocyanin dose (93). Among school-aged children, anthocyanin-rich blueberry products have demonstrated acute cognitive benefits during critical brain developmental periods (93–95,98).

In addition to blueberries, a variety of other anthocyanin-rich fruits have also been explored for their acute neurocognitive benefits, including blackcurrants, cherries, grapes, and plums (89,99–101). Acute supplementation of these anthocyanin-rich fruits have largely been studied among healthy younger and older adults. Containing significant heterogeneity in study design, anthocyanin doses (55.8-937mg), and neurocognitive assessments, drawing comparisons across these studies is challenging. Although these studies produced mixed findings, anthocyanin supplementation trended towards a cognitive benefit among healthy young adults, particularly attention function (100–102). Further well-designed RCTs are warranted to confirm the acute neurocognitive effects of fruit-based anthocyanin-rich supplementation among healthy adults, as well as older adults with cognitive impairments. An important consideration for future studies is the timing of the neurocognitive assessments following anthocyanin supplementation. As plasma anthocyanins and their metabolites reach peak levels at one-to-two and six-hours following ingestion, the presence or absence of a significant effect may be attributed to the timing of the neurocognitive assessment (89).

Table 1.2. Summary of clinical trials examining the effect of acute anthocyanin supplementation on cognitive function

| Product | Acute serve & dose | Study design | Population | Cognitive outcomes | Results[1] | Reference |
|---|---|---|---|---|---|---|
| Blackcurrant juice | 250mL (115mg anthocyanins) | RCT, DB, PC, CO | Healthy young adults (n=9) | Assessed cognitive function according to CogTrack & EEG | No improvement to cognitive performance<br>Small ↑ in reaction times | (102) |
| Blackcurrant extract or juice | 1.66g extract or 142mL juice (344-937mg anthocyanins) | RCT, DB, PC, CO | Healthy young adults (n=36) | Assessed attention function & cognitive flexibility according to a cognitive battery | ↑ accuracy (extract only)<br>↑ reaction time (juice only) | (100) |
| Wild blueberry drink | 200mL (253mg anthocyanins) | RCT, SB, PC | Children aged 7-10 years (n=54) | Assessed executive function & memory function according to AVLT, MANT & reading task | ↑ improve memory & attention<br>No effect on reading measures | (98) |
| Blueberry drink | 200g fresh blueberries (143mg anthocyanins) | RCT, DB, PC,CO | Children aged 8-10 years (n=14) | Assessed cognitive function according to a cognitive battery | ↑ delayed recall<br>No effect on attention or visuospatial memory | (95) |
| Freeze-dried wild blueberry powder | 15 or 30g (127 or 253mg anthocyanins, respectively) | RCT, DB, PC, CO | Children aged 7-10 years (n=21) | Assessed verbal memory, word recognition, response interference & inhibition according to cognitive battery | ↑ immediate recall<br>↑ delayed word recognition<br>↑ accuracy<br>Dose-response model observed | (93) |
| Freeze-dried wild blueberry powder | 30g (253 anthocyanins) | RCT, DB, PC, CO | Children aged 7-10 years (n=21) | Assessed executive function according to MANT | ↑ executive function | (94) |
| Freeze-dried blueberry powder | 30g (579mg anthocyanins) | RCT, DB, PC, CO | Healthy older adults (n=18) | Assessed cognitive function according to a cognitive battery | ↑ immediate word recognition<br>↑ cerebral blood flow | (96) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Grape & blueberry extract | 600mg (unknown anthocyanin dose) | RCT, DB, PC, CO | Healthy young adults (n=30) | Assessed working memory & attention according to a cognitive battery, including RVIP | ↑ sustained attention<br>↑ working memory | (101) |
| Cherry juice | 300mL (55.8mg anthocyanins) | RCT, CO | Young healthy adults (n=6), older adults (n=5), older adults with dementia (n=5) | Verbal learning, memory & executive function according to RAVLT, task-switching task | No effect on cognitive tasks (except tasking-switching test in older adults) | (99) |
| Plum juice | 1 x 300mL or 3 x 100mL (369mg anthocyanins) | RCT, CO | Younger adults (n=12), older adults (n=12) | Assessed cognitive function according to a cognitive battery, including RAVLT, Stroop tasks | No observed effect on cognition in either dose-timing regimen or age group | (89) |

[1] Significant results ($p<0.05$) presented include the effect (i.e. cognitive tool and/or its extrapolated cognitive domain) of anthocyanin supplementation; the cognitive domain was included only when conferred by the author). Abbreviations: RCT, randomised controlled trial; DB, double-blind; PC, placebo-controlled; CO, crossover; SB, single-blind; EEG, electroencephalography; RAVLT, Rey's Auditory Verbal Learning Task; MANT, Modified Attention Network Task; RVIP, rapid visual information processing task.

*Daily anthocyanin supplementation*

In addition to acute supplementation, a variety of studies have explored the effect of daily anthocyanin supplementation on neurocognitive outcomes (Table 1.3). Blueberries again are widely studied in longer-term clinical trials (57,91,103–105). Daily supplementation of a range of blueberry products (concentrate, extract, freeze-dried powder, juice) have been investigated among older adults with or without some degree of cognitive impairment (57,91,103–105). Double-blind and placebo-controlled by design, the RCTs involved daily anthocyanin doses between 1.35-387mg, from 12 weeks to 6 months. Following daily blueberry supplementation, no consistent pattern was evident across cognitive domains, among healthy older adults or older adults with mild cognitive impairments (106). Although these studies produced mixed findings across cognitive tools, two studies have reported improved MRI brain perfusion and/or activation in areas associated with cognitive function (57,91).

A variety of other anthocyanin-rich foods or supplements have also been utilised in daily supplementation clinical trials, including blackcurrant, pine bark extract (rich in proanthocyanidin class of anthocyanins), grapes, and blackberries (59,107–110). Daily supplementation of these anthocyanin-rich products have been investigated among healthy middle-aged adults and older adults (90,92,109–112). Although these studies reported inconsistent results, daily anthocyanin supplementation trended towards supporting neurocognition among healthy populations, particularly memory and attention function. Daily anthocyanin supplementation has also been investigated among older adults with early-to-mild cognitive decline and mild-to-moderate dementia (59,88,108). These populations also demonstrated a cognitive benefit, with one study reporting a greater activation in anterior and posterior regions of the brain's right hemisphere following supplementation of Concord grape juice (355-621mL) for 16 weeks (59).

Only one study has explored the neurocognitive effects of the current study's polyphenol-rich nutrition intervention, known as Ārepa, a black currant-based nootropic drink also containing pine-bark and l-theanine (107). This RCT investigated one week of Ārepa supplementation, on mental performance in a sport setting (107). Cognition was assessed pre- and post-intervention following a standardised training session, finding the blackcurrant drink significantly improved the total score, accuracy, and average time per response score (107).

**Table 1.3.** Summary of clinical trials examining the effect of long-term anthocyanin-rich products on cognitive function

| Product | Daily dose & intervention period | Study design | Population | Cognitive outcomes | Results[1] | Reference |
|---|---|---|---|---|---|---|
| Ārepa beverage | 250mL for 7 days (311mg anthocyanins) | RCT, DB, PC, CO | Sub-elite rugby league players (n=27) | Assessed mental clarity according to the Stroop task | ↑ total cognitive score<br>↑ accuracy & time per response | (107) |
| Wild blueberry powder or purified extract | 500 or 1000mg powder, or 100mg extract for 6 months (1.35, 2.7, or 7mg anthocyanins) | RCT, DB, PC | Healthy older adults (n=122) | Assessed episodic & working memory & executive function according to a cognitive battery, including RAVLT, MANT & Stroop | ↑ episodic memory<br>No effect for working memory & executive function | (103) |
| Wild blueberry juice | 6-9mL/kg for 12 weeks (unknown anthocyanin dose) | RCT, DB, PC | Older adults with early memory deficits (n=9) | Assessed memory function according to the V- PAL & CVLT | ↑ memory function | (104) |
| Freeze-dried blueberry powder | 24g for 12 weeks (unknown anthocyanin dose) | RCT, DB, PC | Healthy older adults (n=37) | Assessed executive function according to the CVLT-II, digit span task, virtual version of the Morris Water Maze & Attention Network Task | ↑ accuracy in task-switching test<br>↓ repetition errors in word list recall test | (105) |
| Blueberry concentrate | 30mL for 12 weeks (387mg anthocyanins) | RCT, DB, PC | Healthy older adults (n=26) | Assessed psychomotor function, visual processing, executive function, verbal, spatial & working memory according to Cogstate battery, MRI | ↑ working memory<br>↑ brain perfusion & activation | (57) |
| Blueberry powder | 12.5g for 16 weeks (unknown anthocyanin dose) | RCT, DB, PC | Older adults with mild cognitive impairment (n=16) | Assessed working memory according to the sequential letter n-back working memory task & functional MRI | ↑ in MRI brain activity<br>No significant effect on working memory | (91) |
| Blackcurrant extract | 600mg for 7 days (210mg anthocyanins) | RCT, DB, PC, CO | Older adults (n=14) | Assessed reaction time, paired associates learning, spatial working memory & rapid visual information processing according to CANTAB | No effect on cognitive function | (110) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pinus radiata bark (Enzogenol® extract) | 960mg for 5 weeks (unknown anthocyanin dose) | RCT, DB, PC | Male middle-aged adults (n=42) | Assessed spatial & object memory, executive processes, attention & processing speed | ↑ speed of response for the immediate recognition & spatial working memory tasks | (109) |
| Concord grape juice | 355mL for 12 weeks (167mg anthocyanins) | RCT, DB, PC, CO | Healthy, middle-aged females (n=25) | Assessed memory, executive function & attention according to a cognitive battery & driving stimulation | ↑ immediate spatial memory<br>↑ driving performance | (92) |
| Concord grape juice | 355-621mL for 16 weeks (unknown anthocyanin dose) | RCT, DB, PC | Older adults with mild cognitive decline (n=21) | Assessed memory function & brain activation according to CVLT-II & MRI | ↓ semantic interference on memory tasks<br>↑ in MRI brain activity | (59) |
| Concord grape juice | 444-621mL for 12 weeks (unknown anthocyanin dose) | RCT, DB, PC | Older adults with early memory decline (n=12) | Assessed memory function according to the CVLT | ↑ verbal learning | (108) |
| Grape seed extract | 250mg for 12 weeks (unknown anthocyanin dose) | RCT, DB, PC | Healthy older adults (n=111) | Assessed cognitive function using MMSE & RBANS | ↑ attention<br>↑ immediate memory & delayed memory | (90) |
| Tart Montmorency cherry concentrate | 60mL for 12 weeks (22.2mg anthocyanins) | RCT, DB, PC | Middle-aged adults (n=50) | Assessed cognitive function according to a cognitive battery | ↑ accuracy in digit vigilance<br>↑ sustained attention | (112) |
| Cherry juice | 200mL for 12 weeks (138mg anthocyanins) | RCT, DB, PC | Older adults with mild-to-moderate dementia (n=49) | Assessed verbal fluency, short-term & long-term memory according to a cognitive battery, including RAVLT | ↑ verbal fluency<br>↑ short-term memory<br>↑ long-term memory | (88) |
| Black chokeberry extract | 90 or 150mg for 24 weeks (16 or 27mg anthocyanins, respectively) | RCT, DB, PC | Healthy, middle-aged, overweight adults (n=101) | Assessed psychomotor speed, attention, & cognitive flexibility according to a cognitive battery | ↑ psychomotor speed<br>No effect for attention or cognitive flexibility | (111) |

[1] Significant results ($p<0.05$) presented include the effect (i.e. cognitive tool and/or its extrapolated cognitive domain) of anthocyanin-rich product compared with placebo; the cognitive domain was included only when conferred by the author. Abbreviations: RCT, randomised controlled trial; DB, double-blind; PC, placebo-controlled; CO, crossover; RAVLT, Rey's Auditory Verbal Learning Task; MANT, Modified Attention Network Task; V-PAL, Verbal Paired Associate Learning Test; CVLT, California Verbal Learning Test; CVLT-II, California Verbal Learning Test 2nd edition; CANTAB, Cambridge neuropsychological test automated battery; MMSE, Mini-Mental State Examination; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.

## 1.5 Research gaps and rationale

This literature review has highlighted the importance of maintaining brain health across the life-course and the potential of polyphenols to do so, including several lines of evidence regarding mechanistic plausibility and efficacy in human clinical trials. Although research to date has trended towards a neurocognitive benefit following anthocyanin intake in particular, many gaps in the literature still remain.

Firstly, there are important research gaps owing to methodological considerations. For example, there are very few long-term polyphenol supplementation studies exploring neurocognition with a cross-over design, increasing the risk of confounding and decreasing the efficacy of comparing the intervention and placebo. There is also an absence of long-term anthocyanin supplementation studies among healthy young and middle-aged adults, with the evidence-base largely focusing on older adults with and without some degree of cognitive impairment. Previous long-term anthocyanin supplementation studies among healthy adults have recruited using flawed convenience sampling methods, including negligence of baseline diet and nutrient status prior to the intervention, disregarding its potential impact of the integrity of the findings (113).

Second, despite the recognised association between stress reactivity, mental health, and cognitive function, comparatively little is known about how anthocyanins may influence how people respond to cognitive stressors. Several lines of evidence point towards the benefits of polyphenols for longer-term measures of mental health (97,102,114–116), however, very few long-term anthocyanin supplementation studies have explored the effect of anthocyanins upon stress reactivity measures. This is an important piece of missing evidence because stress reactivity is an important precedent for the development of mental ill-health outcomes (11). Stress reactivity is an appealing measure in human clinical trials measuring cognition. Not only is stress reactivity an important precedent for mental health more broadly, it can be measured before and after a cognitive stressor with minimal participant burden, for example in cognitively demanding tasks used to measure cognitive performance. Limited evidence exists examining stress reactivity measures in polyphenol intervention studies. A notable double-blind, placebo-controlled RCT examining daily tart Montmorency cherry supplementation (391mg anthocyanins) found the anthocyanin-rich beverage to improve perceived alertness and lower perceived mental fatigue, following a cognitive stressor (112). This presents a significant research gap, warranting future investigation.

Lastly, there are very few human clinical trials utilising blackcurrants as an anthocyanin-rich intervention. Studies have predominately utilised anthocyanin-rich fruits readily available to its region, particularly blueberries, grapes, and cherries. An anthocyanin-rich fruit readily grown in New Zealand is blackcurrants, whereby approximately 6500-9000 tonnes are grown annually (117). Additionally, blackcurrants grown in New Zealand have been reported to have higher anthocyanin content than those grown in other countries, which is likely attributed to the nation's environment with high UV exposure (118). The total anthocyanin content of New Zealand-grown blackcurrants ranges between 336 to 850mg/100mL, compared to blackcurrants grown elsewhere with 170 to 310 mg/100 mL of juice (119). The potential of anthocyanin-rich blackcurrants to influence neurocognition has been significantly under-researched. Approximately four studies to date have explored the acute or long-term effect of blackcurrant supplementation on neurocognitive function, across a variety of populations (100,102,107,110). Ārepa, a commercially available beverage containing New Zealand blackcurrant, was therefore considered a good candidate for a human clinical trial.

Taking into account the research gaps, the LINK study was proposed. Using a cross-over, double-blind randomised control design, the LINK study aimed to investigate the effect of Ārepa, an anthocyanin-rich blackcurrant drink, on neurocognitive outcomes, including stress reactivity and cognitive function. Healthy female adults were recruited by baseline diet status according to a priori screening with the Dietary Screening Tool (DST), with the aim of capturing a wider distribution of diet quality including a balance of 'optimal' and 'sub-optimal' diets.

## 1.6 Thesis objectives

*Primary objective*

To measure the effects of 4-weeks of Ārepa supplementation on stress reactivity to a multi-tasking cognitive stressor compared to placebo in healthy females, including acute changes to subjective anxiety, mood, and stress.

*Secondary objectives*

- To measure the effects of 4-weeks of Ārepa supplementation on cognitive performance compared to placebo in healthy females, including attention, psychomotor function, working memory, and an aggregate multi-tasking score (derived from the cognitive stressor used in the primary objective).
- To measure differences in cognitive performance and stress reactivity responses to the intervention according to diet quality at baseline.

# Chapter 2: Methods & Materials

## 2.1 The LINK Study: polyphenol-rich drink for gut and brain health

The LINK study was a double-blinded, randomised, placebo-controlled cross-over trial. The overarching aim of the LINK study was to investigate the effects of Ārepa (a polyphenol-rich blackcurrant drink) on markers of the gut-brain axis in healthy female adults, including neurocognitive function, biochemical markers, and gut microbiota composition. The study was conducted between August 2022 and January 2023 at the University of Auckland Clinical Research Centre (Faculty of Medical and Health Sciences) in Grafton, Auckland. This study was funded by High Value Nutrition (National Science Challenges, grant #1968), with in-kind product contributed by AlphaGen Ltd.

### 2.1.1 The current study

The research presented in this 90-point Masters thesis is focused on the effect of the polyphenol-rich blackcurrant drink on neurocognitive outcomes, including stress reactivity and cognition. Blood, microbiome, and other well-being (sleep, mood) outcomes are presented in Masters student theses elsewhere.

## 2.2 Recruitment

The LINK study recruited healthy female adults residing within the Auckland region. Recruitment involved advertisements at the University of Auckland campuses, online and print strategies (including social media platforms), and printed flyers. After expressing interest in the trial either through an online form or directly contacting the research team, participants completed an online screening questionnaire. If eligible, participants were invited to an enrolment visit which included a detailed description of the trial and an opportunity to have questions answered before being asked to provide informed consent (Appendix 1 and 2).

## 2.3 Participants

### 2.3.1 Inclusion and exclusion criteria

**Table 2.1** Study inclusion and exclusion criteria

| Inclusion | Exclusion |
|---|---|
| • Females aged between 18-45 years<br>• BMI of 18-30 kg/$m^2$<br>• Not pregnant or intending to become pregnant during the trial<br>• Has access to the internet and a computer, tablet, or smart phone<br>• Agrees not to partake in another interventional clinical research trial 4 weeks prior to, and throughout the duration of the study period<br>• Comprehends and is willing to comply with all study procedures<br>• Willing and able to provide written informed consent | • Treated for anxiety, depression, or psychiatric disorders within the last 2 years<br>• History of neurological disorders (e.g. Parkinson's disease, stroke, epilepsy, serious head trauma), cognitive impairment, cardiovascular diseases or diabetes mellitus requiring medication<br>• Diagnosis of gastrointestinal disorders (e.g. Crohn's disease, ulcerative colitis, coeliac disease), or undergone major gastrointestinal surgery (e.g. ileostomy, colostomy, hemi colectomy) expected to interfere with study outcomes<br>• Medication use likely to interfere with normal digestive processes (e.g. laxatives, proton pump inhibitors)<br>• Antibiotic use within the 4 weeks prior to beginning the intervention<br>• Herbal extract supplement use likely to interfere with mood or cognition, and not willing to cease intake for 4 weeks before and throughout the intervention<br>• Probiotic or prebiotic supplement use, and not willing to cease use for 4 weeks before and throughout the intervention<br>• Self-reported alcohol consumption exceeding a moderate intake (i.e. >15 standard drinks per week)<br>• Regular use of recreational/illicit drugs<br>• Sensitivity to the intervention product, or any of its active/inactive ingredients |

### 2.3.2 Dietary Screening Tool

The Dietary Screening Tool (DST) was used to categorise participants into balanced 'optimal' (n=20) and 'sub-optimal' (n=20) diet groups during recruitment. The DST was originally designed for community-dwelling older adults, but has also been validated in middle-aged adults (120,121). The tool was adjusted to comprise dietary products and brands appropriate to New Zealand, for example Hungry Jacks was replaced with Burger King. The DST comprises of 20 items, including 18 items pertaining to the frequency of consumption of particular foods and two items pertaining to the number of servings consumed (Appendix 3). The DST has a maximum score of 104, with higher scores signifying greater diet quality and lower nutritional risk. Higher DST scores are associated with greater intakes of fruit, vegetables, legumes, nuts, and olive oil, and lower consumption of processed foods, including confectionary, baked goods, sugar-sweetened beverages, and processed meats. Cut-off scores of ≥60 ('optimal') and ≤59 ('sub-optimal') were used, which have identified nutritional risk according to circulating micronutrient status in middle-aged adults (121).

## 2.4 Intervention

Participants received the following daily interventions for four weeks each:

1. Active intervention- Ārepa performance drink (300mL)
2. Control intervention- Placebo beverage (300mL)

The ingredients and nutritional composition of the intervention and control beverages are shown in Table 2.2. The placebo beverage was matched for colour, taste, and macronutrient composition to the Ārepa performance drink, and has been successfully used in a prior RCT (107). The intervention was three months in duration, including four weeks on each intervention arm, and a four-week wash-out period between arms. The intervention and control products were prepared and packaged by Ārepa Ltd, with identical packaging excluding their printed batch codes and expiry dates. The intervention and control beverages were each produced in a single batch and then stored in dark, temperature-controlled rooms before distribution to participants.

### 2.4.1 Randomisation and blinding

**Table 2.2.** Ingredients and nutritional composition of the intervention and control beverage

| | Ārepa performance beverage (300mL) | Control beverage (300mL) |
|---|---|---|
| Ingredients | New Zealand Neuroberry® Blackcurrant Juice, Ultra-filtered Water, New Zealand Apple Juice, Erythritol (Natural Sweetener), Natural Flavours, New Zealand Blackcurrant Extract, L-theanine Suntheanine®, New Zealand Pine Bark Extract Enzogenol®, Natural Acidity Regulator (Citric), Stevia (Natural Sweetener) | New Zealand Apple Juice, Ultra-Purified Water, Erythritol (Natural Sweetener), Natural Flavours, Elderberry Juice Concentrate, Natural Colours, Artificial Flavours, Natural Acidity Regulator (Citric), Ascorbic Acid, Stevia (Natural Sweetener) |
| Energy (kJ) | 155 | 155 |
| Protein (g) | 0.4 | 0.4 |
| Total Polyphenols (mg) | 1297 | 22 |
| Anthocyanins (mg) | 311 | 7 |
| L-theanine (mg) | 200 | 0 |
| Fat total (g) | 0 | 0 |
| Fat Saturated (g) | 0 | 0 |
| Carbohydrate (g) | 23 | 23 |
| Sugars (g) | 14.8 | 14.8 |
| Sodium (g) | 8 | 8 |
| Enzogenol® (mg) | 150 | 0 |
| Vitamin C (mg) | 90 | 90 |

The sequence of intervention (active then placebo, or placebo then active) was randomised with an equal allocation ratio balanced for DST category, with both participants and investigators blinded to the identity of treatments for the duration of the trial and data analysis. The randomisation sequence was computer generated by an independent researcher (122).

## 2.5 Procedures

### 2.5.1 Participant timeline

Figure 2.1 demonstrates an overview of the study timeline and procedures, including participant recruitment, study visits, and intervention arms. This section describes the procedures used in the entire LINK study to give context to participant burden, rather than the methodology specifically relevant to this section. Throughout the trial, participants attended the Clinical Research Centre for five study visits: enrolment, weeks 1 and 4 (arm 1), weeks 9 and 12 (arm 2).

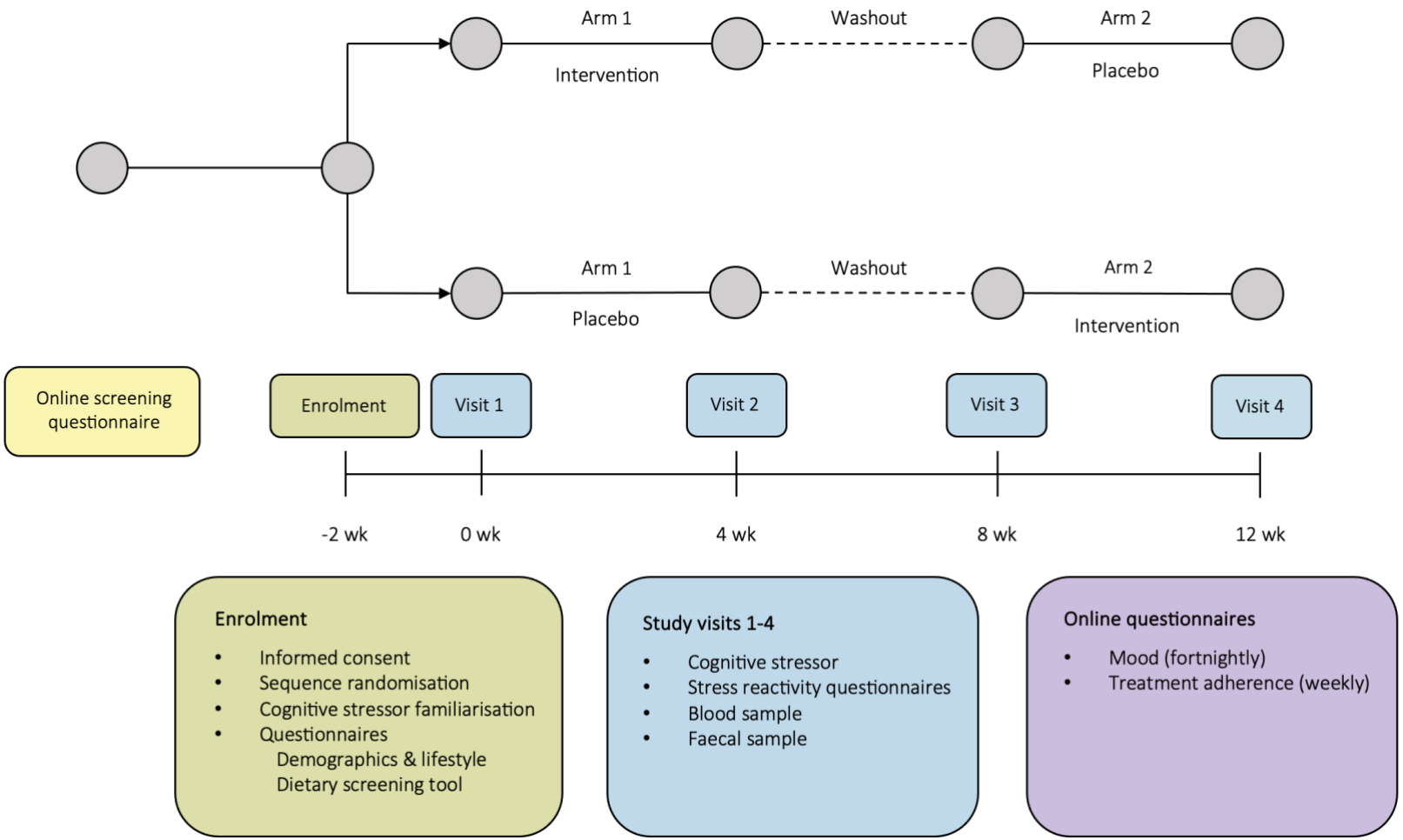
*Enrolment visit*

At the enrolment visit, participants completed baseline questionnaires and a familiarisation session with the Purple-MTF to minimise the impact of learning effects in subsequent study visits. Participants were given a stool sample collection kit and were provided with detailed instructions regarding delivery of their collected stool sample at subsequent study visits. Participants were randomly assigned an intervention sequence once the enrolment visit was completed.

*Study visit 1-4*

The intervention period began approximately two weeks following the enrolment visit. All four study visits were scheduled at the same time for each participant. Prior to each study visit, participants were instructed to fast overnight, as well as abstain from alcohol and caffeine in the 24 hours and 12 hours preceding each study visit, respectively. The following morning, participants arrived between 7am- 9am for their study visit. Upon arrival, a stool sample was collected and transferred to a -80°C freezer for storage. Next, participants completed the Purple-MTF, taking approximately 20 minutes to complete. Immediately before and after the Purple-MTF, both the State-Trait Anxiety Inventory: State Subscale (STAI-S) and Visual Analogue Mood Scales (VAMS) were completed. Following the neurocognitive testing, a fasted blood sample and anthropometric data were collected. At each clinic visit, an assessment for adverse events was performed. Each intervention arm was separated by a four-week washout period in which no study beverage was consumed, and participants were to maintain their usual diet and lifestyle. Participants collected their allocated intervention drinks at study visit 1 and 3, which were prepared by an unblinded researcher. To increase study reliability and minimise study heterogeneity, the same researcher (Dr Nicola Gillies) completed all data collection using the same equipment.

Figure 2.1. Simplified schematic diagram of study procedures



## 2.6 Data Collection

### 2.6.1 Cognitive stressor: Multi-Tasking Framework (MTF)

The MTF (Purple Research Solutions, Northumbria, UK) is a computerised, multi-tasking tool. The Purple-MTF was utilised in the study, concurrently assessing cognitive performance and eliciting psychological stress. The Purple-MTF has been shown to increase subjective and physiological measures of stress (123). Unique amongst lab stressors, the Purple-MTF can be repeated on several occasions with limited learning effect (124) and has demonstrated its suitability for use in cross-over trials (125). The MTF comprises of four concurrent cognitive and psychomotor tasks, presented in quadrants of a split screen (Figure 2.2). A 20-minute version of the MTF was utilised in the study, requiring participants to simultaneously perform four tasks: 'mental arithmetic', 'Stroop colour-word', 'memory search' and 'visual monitoring' (123). A description and scoring of each MTF domain are found in Table 2.3.

Participants were responsible for attending to the four different tasks concurrently, whilst also monitoring the counter displayed in the centre of the quadrants, revealing their aggregate score. This aggregate score is determined by both the speed and accuracy of the responses across the four tasks. Throughout the MTF, participants were monitored by research staff at regular intervals, positioned within peripheral vision to seemingly monitor performance, and heighten performance anxiety.

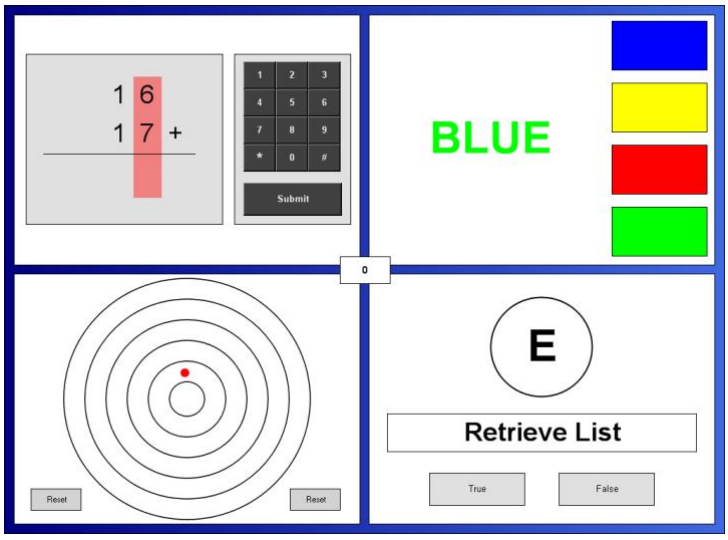
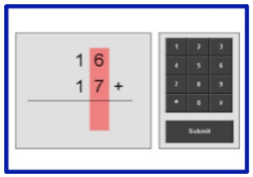
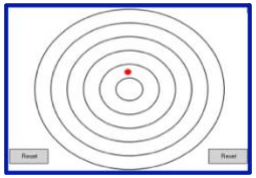



Figure 2.2. Screen layout of the multi-tasking tool

Table 2.3. The domains of the multi-tasking framework

| MTF domain | Description | Picture example | Scoring |
|---|---|---|---|
| Mental arithmetic | The mental arithmetic presents an addition equation of the numbers displayed on screen, requiring participants to enter their answer using the number pad. |  | +10 points are allocated for correct responses and -10 points for incorrect responses or timeouts. |
| Stroop | The Stroop task displays colour words ("blue", "green", "red", or "yellow") in text which are presented in one of the four corresponding colours. Within 20 seconds, participants are required to indicate the font colour by selecting the correct colour displayed in the panel. |  | |
| Memory word search | The memory search task displays a set of four letters which disappear after four seconds. Next, a single probe letter is presented, whereby participants have 15 seconds to decide whether the probe letter appeared in the original set of four letters by selecting the true or false button. |  | |
| Visual monitoring task | The visual monitoring task presents a red dot which moves outwards through a series of concentric circles. Participants are required to select the reset button before the red dot move beyond the outermost circle. |  | A maximum of +10 points if the reset button is selected while the red dot is within the outermost circle, through to +2 points if within the innermost circle. Points are lost at a rate of -10 points for every 0.5 seconds where the red dot has reached the boundary of the outermost circle without being reset. |

### 2.6.2 Assessment of stress reactivity to a cognitive stressor: stress, mood, and anxiety

Participant's stress reactivity was assessed through questionnaires completed immediately before and after completing the Purple-MTF cognitive stressor. Stress reactivity was comprised of anxiety, mood, and stress measures, including the STAI-S (**Appendix 4a**) and VAMS (**Appendix 4b**).

*Visual Analogue Mood Scales*

The Bond-Lader VAMS comprises 16 visual analogue items with antonyms at either end of a horizonal 100mm line, for example 'tense' and 'relaxed'. For each item, participants were required to mark on the line according to the way they felt in that moment. The distance from the negative antonym was measured in millimeters (mm), with the average score from the combined scales used to compute a score for the three mood dimensions. The scores were combined to form three mood dimensions, including 'alert', 'calm', and 'contented'. The VAMS questionnaire has been utilised extensively in research involving on psychopharmacological interventions, with changes observed following the completion of the Purple-MTF (123).

*Stress and Fatigue Visual Analogue Mood Scales*

Two additional measures of subjective stress and fatigue were included to the original VAMS form (126,127). Each item of stress and fatigue included the words 'extremely' and 'not at all' at either end of a horizontal 100mm line. Like the Bond-Lader scales, items were scored using the distance from the 'not at all' end.

*State-Trait Anxiety Inventory: State Subscale*

The STAI-S questionnaire is commonly used in both clinical and research settings and measures anxiety in the present emotional state (128). The STAI-S comprises of 20 items, each scored on a 4-point Likert scale (1= not at all, 2= somewhat, 3= moderately so, 4= very much so). Participants were instructed to respond with the statement which best describes their present feelings. The STAI-S scores were calculated on a scale of 20-80, whereby higher scores reflect a higher present-state anxiety.

### 2.6.3 Cognitive Performance

The Purple-MTF produces a concomitant assessment of memory, psychomotor, and attentional performance. In the study, cognitive performance was assessed through the scores of the four individual tasks, as well as the aggregate score, which demonstrates the ability to multi-task and reflects executive functioning.

### 2.6.4 Dietary intake

Participants were required to complete one 3-day food record prior to their first study visit. Participants were given detailed instructions on how to accurately complete the food record, and each food record was reviewed for completeness by Dr Nicola Gillies prior to dietary analysis. All food records were solely analysed by the author (JM) to maintain consistency of data entry. The food records were assessed to ascertain caloric, macronutrient, micronutrient, fibre and sugar intakes at baseline. Software utilised to input and analyse food record was FoodWorks 10 Professional (Xyris™). This nutritional analysis software uses the most recent and comprehensive food composition data for Australia and New Zealand.

As the food records were entered into FoodWorks, a record of assumptions made for any insufficient details regarding the type, brand, quantity of ingredients or preparation of meals was documented. The New Zealand FOOD files database was utilised for fresh produce items, including fruit and vegetables, meat, poultry, and dairy products. A combination of New Zealand FOOD files and Australian Food Composition databases were utilised for packaged goods. Selected at random, 10% of the participant food records were crosschecked against the dietary intakes inputted into FoodWorks by another student dietitian (ER) working on this research project. If an ingredient, product, or meal was not present in the New Zealand FOOD files or Australian Food Composition databases, the next best item was selected.

## 2.7 Compliance to treatment

The compliance to treatment was monitored using online forms during intervention periods. Each participant was instructed to record the number of beverages (out of the total seven) consumed each week. If less than seven drinks were consumed, research staff recorded the participant's reasoning. Adherence to treatment was recorded as a cumulative score (% of beverages consumed out of the total 28 provided). Minimum adherence requirements were defined a-priori as 80%. Participants were also asked whether they made any changes to their usual dietary and lifestyle behaviours over the previous week. All participant responses were reviewed weekly by research staff to ensure participants adhered to the intervention.

## 2.8 Statistical analysis

### 2.8.1 Power calculation

Power calculations for the LINK study are based on measures of stress as the primary endpoint. After four weeks of intervention with equivalent doses of constituent bio-actives in the Ārepa performance drink (200mg L-theanine), differences in stress-related symptom responses have been reported between intervention (-3.37 ± 8.13) and placebo (0.77 ± 7.52) arms (129). According to this data, a sample size of 35 participants is estimated to be sufficient to produce a between-group difference of 20% to identify significant differences at a level of 5%. A sample size of at least 40 was selected, allowing for a drop-out rate not exceeding 15%.

### 2.8.2 Data analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 29.0.1.0 (171), with statistical significance defined as $p<0.05$. Data analysis was conducted on a "per protocol" basis, excluding the two participants who did not complete both intervention arms. All other participants met compliance requirements of 80% and were included in the analysis. Missing data was treated with mean data imputation where possible, which for this dataset included one missing datapoint for the STAI-S questionnaire. Two participants had corrupted datafiles from the MTF software and on consultation with a biostatistician were not included in analyses for MTF outcomes but were included in other analyses.

Descriptive statistics of the study sample were generated. The mean and standard deviation were calculated for continuous variables, and frequency and percentage for categorical variables. Participant characteristics were presented for the total cohort and were also compared between the sequence allocations.

Differences in changes in the neurocognitive measures from pre- to post-intervention between intervention groups were analysed using paired t-tests. Sub-group analyses were conducted to investigate differences in response according to diet group ('optimal' v 'sub-optimal'). Sensitivity analyses were also conducted to investigate the effect of intervention sequence (placebo > active v active > placebo) on treatment effects. Data visualization was generated using GraphPad Prism Version 10 (131).

### 2.8.3 Ethical considerations

The clinical trial was not initiated until approval by Institutional Review Board/Ethics Committee (IRB/EC). The LINK study was approved by the Health and Disability Ethics Committee on the 27/05/2022 (reference number: 2022 EXP 12513). Those interested in the study were provided detailed information on the trial and participant requirements. In accordance with the International Conference on Harmonization (ICH) guidelines, properly executed written informed consent was acquired from each participant. Formal induction into the trial was completed after the participant provided informed written consent. The researchers ensured the trial was conducted in compliance with the Declaration of Helsinki, including relevant institutional regulations. The trial was pre-registered with the Australian New Zealand Clinical Trials Registry (ACTRN12622000850774).

# Chapter 3: Results

## 3.1 Participant characteristics

Out of 110 potential participants assessed for eligibility, a total of 40 participants were recruited into the study. Two participants were lost to follow-up, with the remaining 38 participants being included in the final analysis (Figure 3.1). A further two of these 38 participants had corrupted datafiles from the MTF software, therefore were excluded in the MTF analyses but were included in other analyses (Figure 3.1).

Overall, the study cohort is a generally healthy population who are well-educated. Participants predominately had alcohol intakes within recommended limits, body mass index within a healthy range, high or moderate exercise levels, and excellent or good self-reported health status, and the majority had obtained University qualifications. It should be noted that the study population is ethnically biased, with most participants identifying as New Zealand European. The participant demographics were generally well-matched between the two sequence allocation groups, with the exception of self-reported alcohol consumption and exercise levels. Compared to participants receiving the placebo first, alcohol intakes were higher in the group allocated to receive the active intervention first. In the group allocated to the placebo first, a greater proportion of participants expressed having high self-reported levels of exercise. Whereas, in the group allocated to the active intervention first, a greater proportion of participants had moderate self-reported levels of exercise. A summary of demographics for the total study population at baseline is outlined in Table 3.1.

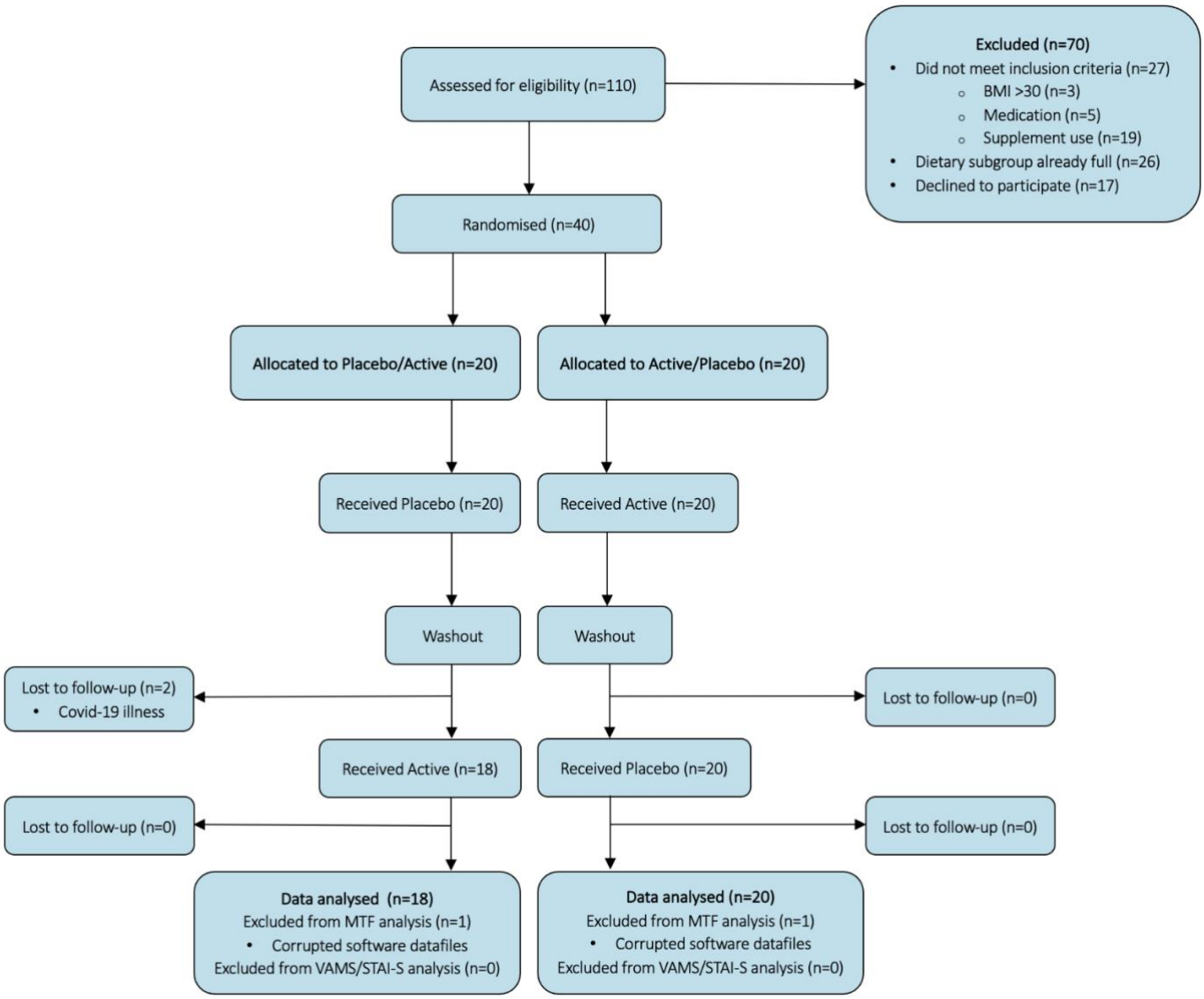



Figure 3.1. CONSORT flow diagram of the current study illustrating participant involvement from recruitment through to data analysis

Study flow diagram for the LINK study analyses presented in this thesis according to the Consolidated Standards of Reporting Trials (CONSORT).

Table 3.1. LINK Study participant characteristics

| | Total sample (n=38) | Placebo/Active (n=18) | Active/Placebo (n=20) |
|---|---|---|---|
| **Age** | 29.8 ± 7.1 | 29.5 ± 6.0 | 30.2 ± 8.0 |
| **Education** | | | |
| NCEA L3 or equivalent | 3 (7.9%) | 1 (5.6%) | 2 (10.0%) |
| University- undergraduate | 18 (47.4%) | 8 (44.4%) | 10 (50.0%) |
| University- postgraduate | 17 (44.7%) | 9 (50.0%) | 8 (40.0%) |
| **Ethnicity[1]** | | | |
| European | 24 (63.2%) | 12 (66.7%) | 12 (60%) |
| Asian | 5 (13.2%) | 2 (11.1%) | 3 (15%) |
| Latin American | 2 (5.3%) | 1 (5.6%) | 1 (5%) |
| Middle Eastern | 1 (2.6%) | 1 (5.6%) | 0 (0.0%) |
| African | 1 (2.6%) | 0 (0.0%) | 1 (5%) |
| NZ European, Māori | 2 (5.3%) | 0 (0.0%) | 2 (10%) |
| Chinese, Other European | 1 (2.6%) | 1 (5.6%) | 0 (0.0%) |
| NZ European, Singaporean | 1 (2.6%) | 1 (5.6%) | 0 (0.0%) |
| Singaporean Eurasian, Malaysian Chinese | 1 (2.6%) | 0 (0.0%) | 1 (5%) |
| **Employment** | | | |
| Full time | 24 (63.2%) | 10 (55.6%) | 14 (70.0%) |
| Part time | 3 (7.9%) | 2 (11.1%) | 1 (5.0%) |
| Studying | 11 (28.9%) | 6 (33.3%) | 5 (25.0%) |
| **Alcohol[2]** | 2.6 ± 2.8 | 1.9 ± 2.3 | 3.2 ± 3.0 |
| **BMI** | 22.8 ± 3.3 | 22.1 ± 1.9 | 23.3 ± 4.1 |
| **DST score** | 65.8 ± 12.6 | 65.9 ± 12.9 | 65.7 ± 12.6 |
| **DST group** | | | |
| Optimal | 19 (50.0%) | 9 (50.0%) | 10 (50.0%) |
| Sub-optimal | 19 (50.0%) | 9 (50.0%) | 10 (50.0%) |
| **Exercise[3]** | | | |
| High | 21 (56.8%) | 12 (70.6%) | 9 (45.0%) |
| Moderate | 13 (35.1%) | 3 (17.6%) | 10 (50.0%) |
| Low | 3 (8.1%) | 2 (11.8%) | 1 (5.0%) |
| **Self-rated health** | | | |
| Excellent | 14 (36.8%) | 7 (38.9%) | 7 (35.0%) |
| Very good | 15 (39.5%) | 8 (44.4%) | 7 (35.0%) |
| Good | 8 (21.1%) | 3 (16.7%) | 5 (25.0%) |
| Fair/poor | 1 (2.6%) | 0 (0.0%) | 1 (5.0%) |
| **Supplements[4]** | | | |
| Yes | 11 (28.9%) | 6 (33.3%) | 5 (25.0%) |
| No | 27 (71.1%) | 12 (66.7%) | 15 (75.0%) |

Data is presented as mean ± standard deviation for continuous variables or n (%) for categorical variables. [1]Ethnicity is classified using Te Whatu Ora level 1 ethnic codes (131). [2]Alcohol refers to self-reported number of standard drinks per week. [3]Exercise refers to self-reported amount of exercise that participants typically engage in according to the International Physical Activity Questionnaire (IPAQ) – Short Form. Note one participant did not complete the exercise questionnaire. [4]Supplements refers to usual supplement intake, ending four weeks prior to the trial. Abbreviations: BMI, Body Mass Index; DST, Dietary Screening Tool; NCEA, National Certificate of Educational Achievement.

## 3.2 Dietary data

A comparison of group average nutrient intakes to adequate intake (AI) or estimated average requirements (EAR) was conducted to provide context of the dietary adequacy of the cohort. Overall, the dietary data further reinforces the study population as a healthy cohort. Group average nutrient intakes were within recommended ranges for most nutrients, with the exception of calcium and sodium. The EAR (women aged 19-50 years) for calcium is 840mg/day (132). However, the mean calcium intakes were nearing requirements at 799mg/day for the total cohort. In addition, the total cohort exceeded the recommended sodium limit of <2000mg/day (133).

A series of unpaired t-tests revealed no significant differences in nutrient intakes between the 'optimal' and 'sub-optimal' dietary groups, including fibre intake. This finding contrasts those previously reported in a cohort of middle-aged Australian adults, in which the DST was able to differentiate fibre intakes between the 'optimal' and 'sub-optimal' diet groups (134,135). A summary of the dietary data for the entire study cohort, 'optimal' diet group, and 'sub-optimal' diet group is outlined in Table 3.2.

Table 3.2. Dietary data for the entire cohort and DST groups

| | EAR or AI[1] | Total sample (n=38) | Optimal (n=19) | Sub-optimal (n=19) | *p* |
|---|---|---|---|---|---|
| Energy (kJ) | - | 7985 ± 1897 | 7927 ± 1786 | 8042 ± 2050 | 0.855 |
| Protein (g) | - | 81 ± 20 | 78 ± 18 | 84 ± 21 | 0.337 |
| kJ from protein (%) | - | 18 ± 4 | 17 ± 2 | 19 ± 5 | 0.231 |
| Total fat (g) | - | 87 ± 29 | 84 ± 26 | 90 ± 31 | 0.500 |
| kJ from fat (%) | - | 40 ± 8 | 39 ± 6 | 42 ± 9 | 0.293 |
| Saturated fat (g) | - | 32 ± 15 | 29 ± 9 | 34 ± 20 | 0.319 |
| kJ from saturated fat (%) | - | 15 ± 5 | 14 ± 3 | 16 ± 7 | 0.304 |
| Polyunsaturated fat (g) | - | 15 ± 6 | 14 ± 6 | 15 ± 6 | 0.553 |
| Monounsaturated fat (g) | - | 32 ± 11 | 32 ± 13 | 31 ± 9 | 0.692 |
| Total carbohydrate (g) | - | 183 ± 64 | 189 ± 51 | 177 ± 76 | 0.586 |
| kJ from carbohydrate (%) | - | 38 ± 10 | 40 ± 6 | 37 ± 13 | 0.505 |
| Sugar (g) | - | 74 ± 27 | 76 ± 20 | 72 ± 34 | 0.681 |
| Fibre (g) | 25 | 29 ± 13 | 31 ± 11 | 27 ± 15 | 0.359 |
| Alcohol (g) | - | 3.5 ± 5.5 | 3.4 ± 5.5 | 3.6 ± 5.6 | 0.930 |
| Calcium (mg) | 840 | 799 ± 297 | 791 ± 287 | 806 ± 314 | 0.878 |
| Iodine (µg) | 100 | 121 ± 127 | 100 ± 48 | 142 ± 174 | 0.315 |
| Iron (mg) | 8.0 | 12.2 ± 4.4 | 12.9 ± 5.2 | 11.6 ± 3.4 | 0.376 |
| Magnesium (mg)[2] | 255, 265 | 357 ± 130 | 373 ± 122 | 341 ± 139 | 0.448 |
| Phosphorus (mg) | 580 | 1333 ± 328 | 1333 ± 303 | 1334 ± 361 | 0.933 |
| Potassium (mg) | 2800 | 3192 ± 1157 | 3271 ± 1067 | 3112 ± 1264 | 0.676 |
| Selenium (µg) | 50 | 56 ± 26 | 56 ± 26 | 57 ± 25 | 0.890 |
| Sodium (mg) | 460-920 | 2331 ± 902 | 2151 ± 769 | 2512 ± 1006 | 0.221 |
| Zinc (mg) | 6.5 | 9.8 ± 2.7 | 9.2 ± 2.4 | 10.4 ± 2.9 | 0.181 |
| Vitamin A (µg) | 500 | 1063 ± 1048 | 958 ± 418 | 1168 ± 1436 | 0.545 |
| Beta carotene (µg) | - | 3850 ± 2681 | 4224 ± 2447 | 3476 ± 2914 | 0.398 |
| Thiamin (mg) | 0.9 | 1.2 ± 0.7 | 1.1 ± 0.5 | 1.3 ± 0.8 | 0.373 |
| Riboflavin (mg) | 0.9 | 1.6 ± 0.7 | 1.5 ± 0.4 | 1.7 ± 0.8 | 0.246 |
| Niacin (mg) | 11 | 17.3 ± 6.6 | 16.9 ± 6.1 | 17.6 ± 7.2 | 0.740 |
| Vitamin $B_6$ (mg) | 1.1 | 2.2 ± 1.1 | 2.2 ± 0.7 | 2.3 ± 1.5 | 0.790 |
| Folate DFE (µg) | 320 | 420 ± 180 | 394 ± 116 | 446 ± 227 | 0.386 |
| Vitamin $B_{12}$ (µg) | 2.0 | 3.2 ± 2.4 | 2.7 ± 0.7 | 3.8 ± 3.3 | 0.143 |
| Vitamin C (mg) | 30 | 107 ± 116 | 118 ± 91 | 97 ± 139 | 0.579 |
| Vitamin E (mg) | 7.0 | 11.1 ± 6.1 | 11.5 ± 4.6 | 10.7 ± 7.5 | 0.694 |
| Caffeine (mg) | - | 178 ± 322 | 181 ± 349 | 174 ± 303 | 0.948 |

[1] The group average nutrient intakes were compared against the EAR or AI for the study population; all nutrient reference values are an EAR, except for fibre, potassium, sodium, and vitamin E which are an AI (132). [2]Magnesium 255mg/day (19-30 yr), 265mg/day (31-50yr) (132). Abbreviations: AI, Adequate Intake; DFE, Dietary Folate Equivalents; EAR, Estimated Average Requirement.

## 3.3 Cognitive performance according to the Purple-MTF

The study found a positive effect of daily Ārepa supplementation on cognitive performance. The change in total MTF scores from baseline to follow-up was greater for the active treatment (+1689) when compared to the placebo (+859), with a p-value nearing statistical significance (p= 0.052). Similarly, the change in MTF search scores from baseline to follow-up was also greater for the active treatment (+955) when compared to the placebo (+425), with a p-value nearing statistical significance (p= 0.057). Daily Ārepa supplementation did not have any effect on mental arithmetic, Stroop or tracking tasks compared to placebo ($p$ >0.05) (**Appendix 5**). A summary of the Purple-MTF task performance scores at baseline and follow-up according to treatment and diet group is outlined in **Table 3.3** and a summary of Purple-MTF total performance change scores is presented in **Figure 3.2**.

### 3.3.1 Exploratory analyses: effect of baseline diet quality on cognitive responses to intervention

To consider the effects of baseline diet quality on neurocognitive responses, we separated 'sub-optimal' and 'optimal' diet groups and repeated the above analysis. Here, we see that the 'sub-optimal' diet group demonstrated improvements in total MTF scores (active change score= +2108, placebo change score = +594, p= 0.028) and MTF search scores (active change score = +1154, placebo change score = +127, p= 0.022) when the active treatment was compared to the placebo, but the same response was not seen in the 'optimal' diet group (total score, p= 0.774; search score, p=0.921).

### 3.3.2 Sensitivity analysis

A sensitivity analysis was conducted with the aim of capturing participants that were following tasks correctly according to instructions and involved in genuine multitasking. Participants were required to achieve at least 25% accuracy for each MTF domain and attend to at least 75% of the stimuli presented. Consequently, the MTF results of seven participants were excluded in this sensitivity analysis. In this sensitivity analysis we see a positive effect of daily Ārepa supplementation on the mental arithmetic task compared to placebo (p=0.039). The complete MTF sensitivity analysis is presented in **Table 3.4**. Another sensitivity analysis according to diet group was not conducted due to a limited sample size.

Table 3.3. Purple-MTF task scores at baseline and follow-up for placebo and active groups

| | | Placebo | | | | Active | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Subgroup | n | Baseline | Follow-up | Δ | n | Baseline | Follow-up | Δ | *p* |
| Total | All | 36 | 7202 ± 2533 | 8061 ± 2203 | 859 ± 1528 | 36 | 6534 ± 2649 | 8222 ± 2534 | 1689 ± 1868 | 0.052 |
| | Optimal | 18 | 6532 ± 2102 | 7656 ± 1854 | 1123 ± 1083 | 18 | 6466 ± 2037 | 7735 ± 2321 | 1269 ± 1539 | 0.774 |
| | Sub-optimal | 18 | 7872 ± 2800 | 8466 ± 2492 | 594 ± 1868 | 18 | 6602 ± 3207 | 8710 ± 2707 | 2108 ± 2108 | 0.028* |
| Maths | All | 36 | 441 ± 355 | 502 ± 311 | 60 ± 244 | 36 | 441 ± 295 | 498 ± 321 | 58 ± 172 | 0.956 |
| | Optimal | 18 | 380 ± 435 | 489 ± 357 | 109 ± 309 | 18 | 389 ± 211 | 496 ± 273 | 107 ± 184 | 0.984 |
| | Sub-optimal | 18 | 503 ± 248 | 514 ± 266 | 12 ± 149 | 18 | 493 ± 359 | 501 ± 371 | 8 ± 147 | 0.946 |
| Stroop | All | 36 | 2898 ± 1655 | 3283 ± 1524 | 385 ± 809 | 36 | 2633 ± 1974 | 3267 ± 1850 | 635 ± 1225 | 0.261 |
| | Optimal | 18 | 2868 ± 1001 | 3136 ± 1099 | 268 ± 494 | 18 | 2766 ± 1092 | 3086 ± 1238 | 320 ± 830 | 0.837 |
| | Sub-optimal | 18 | 2928 ± 2153 | 3430 ± 1878 | 502 ± 1036 | 18 | 2499 ± 2606 | 3449 ± 2332 | 949 ± 1480 | 0.230 |
| Tracking | All | 36 | 438 ± 92 | 427 ± 136 | -11 ± 142 | 36 | 404 ± 268 | 445 ± 57 | 42 ± 264 | 0.203 |
| | Optimal | 18 | 424 ± 122 | 448 ± 76 | 24 ± 96 | 18 | 363 ± 376 | 450 ± 60 | 86 ± 371 | 0.404 |
| | Sub-optimal | 18 | 452 ± 48 | 406 ± 177 | -47 ± 173 | 18 | 444 ± 53 | 441 ± 56 | -3 ± 42 | 0.279 |
| Search | All | 36 | 3424 ± 1738 | 3849 ± 2068 | 425 ± 1078 | 36 | 3057 ± 1932 | 4011 ± 2050 | 955 ± 952 | 0.057 |
| | Optimal | 18 | 2859 ± 1260 | 3582 ± 1187 | 723 ± 785 | 18 | 2948 ± 1236 | 3703 ± 1540 | 755 ± 963 | 0.921 |
| | Sub-optimal | 18 | 3989 ± 1988 | 4116 ± 2692 | 127 ± 1260 | 18 | 3165 ± 2476 | 4319 ± 2467 | 1154 ± 925 | 0.022* |

Purple-MTF scores are presented as mean ± standard deviations. Δ indicates mean change score between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing placebo to active intervention change scores, $*p < 0.05$. A higher score is indicative of a favourable neurocognitive result, whereas a lower score is indicative of a poorer neurocognitive result.

**Table 3.4.** MTF sensitivity analysis in total study population (n=29)

| | Placebo | | | Active | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Follow-up | Δ | Baseline | Follow-up | Δ | *p* |
| **Total** | 7253 ± 2455 | 8076 ± 2228 | 824 ± 1207 | 6746 ± 1823 | 8116 ± 2266 | 1370 ± 1435 | 0.150 |
| **Maths** | 521 ± 267 | 539 ± 325 | 18 ± 132 | 474 ± 310 | 568 ± 308 | 94 ± 161 | 0.039* |
| **Stroop** | 3139 ± 1273 | 3482 ± 1274 | 342 ± 588 | 3026 ± 1019 | 3452 ± 1283 | 427 ± 750 | 0.654 |
| **Tracking** | 431 ± 100 | 438 ± 110 | 7 ± 122 | 399 ± 297 | 453 ± 55 | 55 ± 293 | 0.333 |
| **Search** | 3162 ± 1385 | 3617 ± 1340 | 456 ± 869 | 2848 ± 1051 | 3643 ± 1422 | 794 ± 918 | 0.179 |

Purple-MTF scores are presented as mean ± standard deviations. Δ indicates mean change score between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing placebo to active intervention change scores, *$p < 0.05$. A higher score is indicative of a favourable neurocognitive result, whereas a lower score is indicative of a poorer neurocognitive result. *Participants were excluded from sensitivity analyses for attending to less than 75% of the stimuli presented in the Stroop (n=1), maths (n=2), and word search (n=1) domains, or acheiving <25% accuracy in the Stroop (n=2) or maths (n=2) domain at baseline or follow-up.*

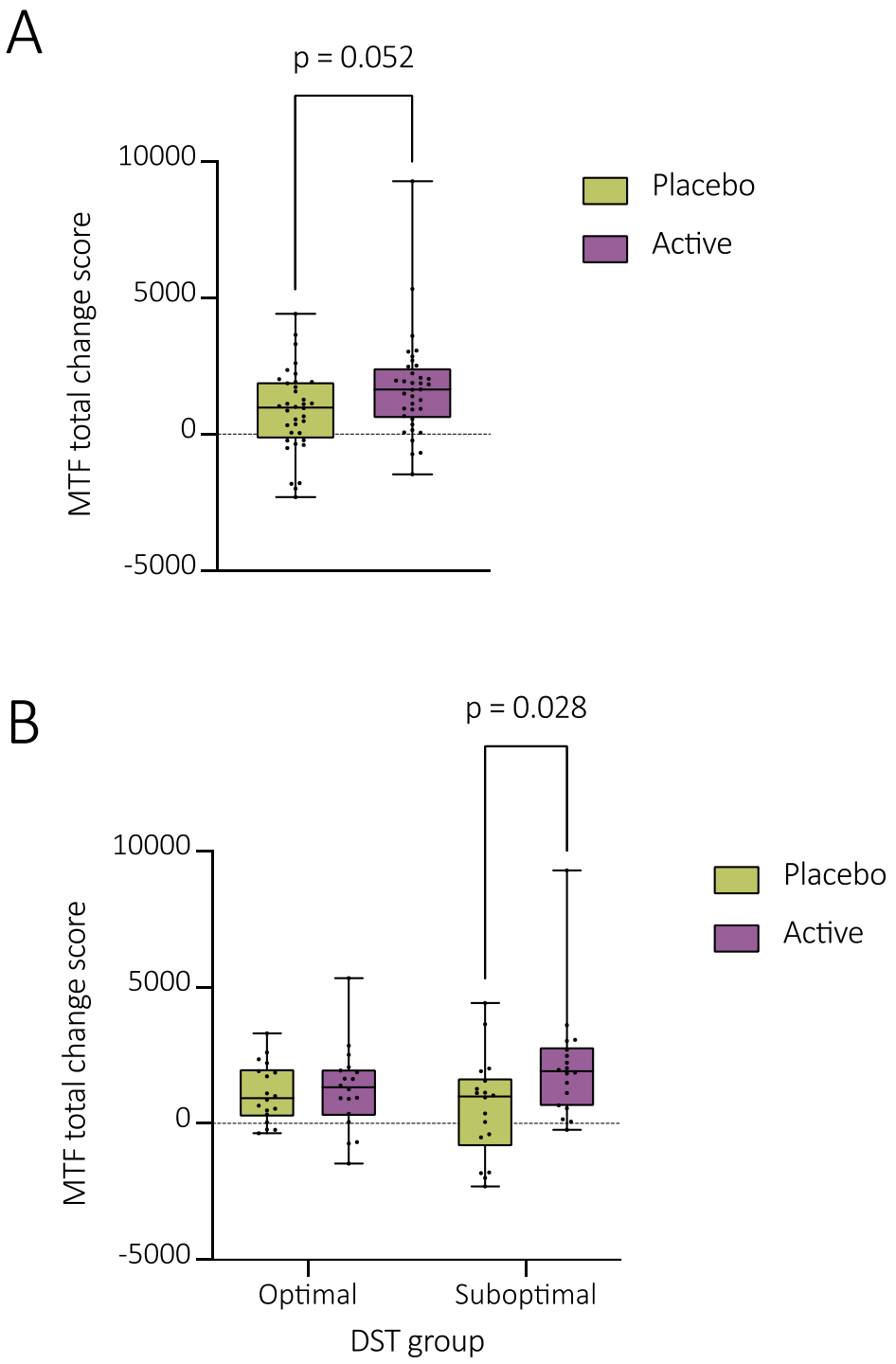



**Figure 3.2.** Effect of treatment on change in MTF total scores

Data presented for A) Total change scores, and B) Total change scores by DST group

The MTF total change score indicates the mean total change score between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing the placebo to active intervention change scores.

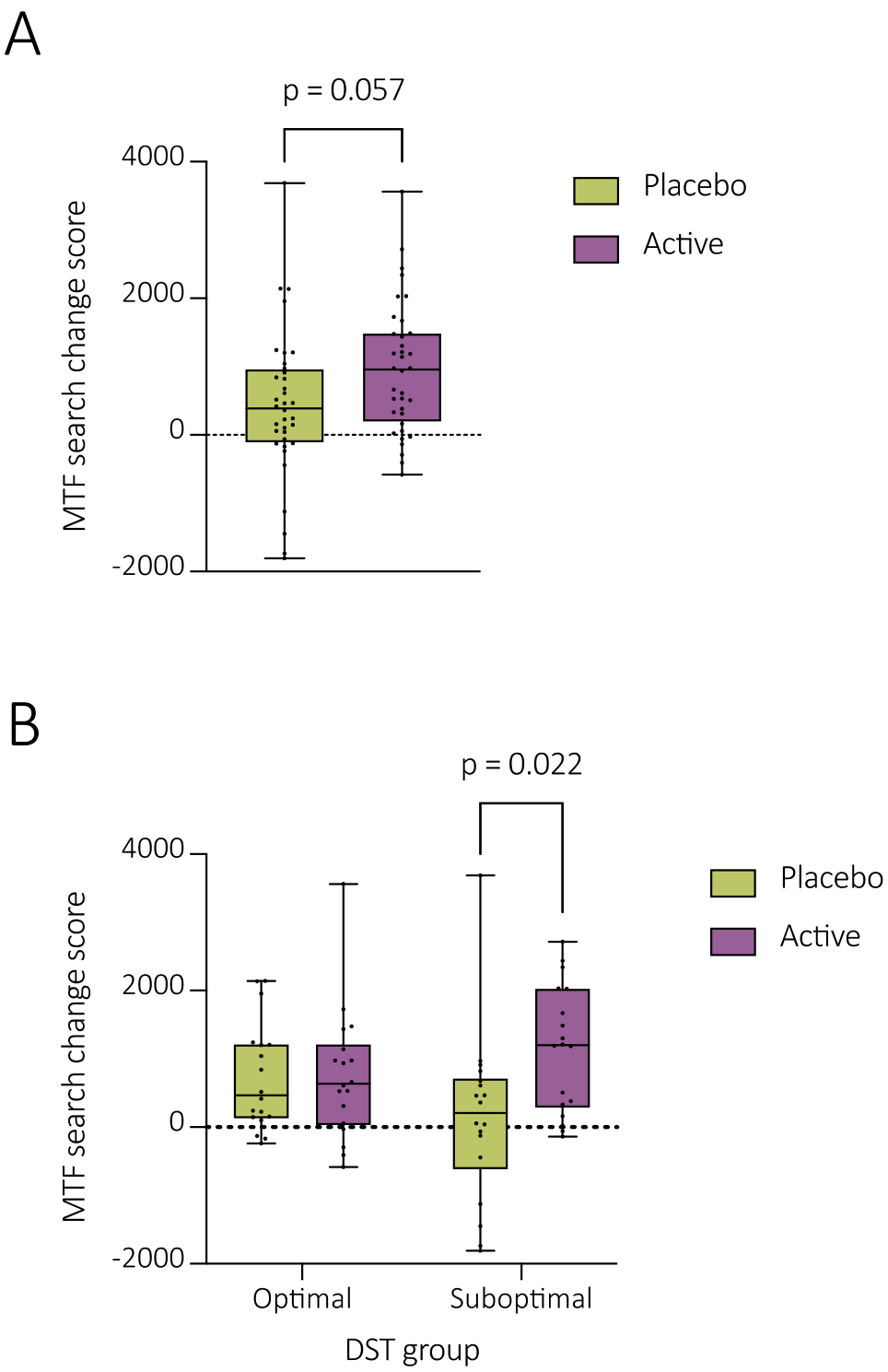



**Figure 3.3.** Effect of treatment on change in MTF search scores

Data presented for A) Search, and B) Search by DST group

The MTF search change score indicates the mean search change score between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing the placebo to active intervention change scores.

## 3.4 Stress reactivity

The study found no positive effect of daily Ārepa supplementation on all measures of stress reactivity when compared to the placebo in the total study population, or in analyses separating 'sub-optimal' and 'optimal' diet groups. A summary of the stress reactivity scores at baseline and follow-up for both treatments (placebo and active) is outlined in **Table 3.5**, and summary of stress reactivity change scores for both the placebo and active treatment is presented in **Figure 3.4**.

Table 3.5. Stress reactivity scores at baseline and follow-up for placebo and active groups

| | | Placebo | | | | Active | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Subgroup | n | Baseline | Follow-up | Δ | n | Baseline | Follow-up | Δ | *p* |
| **Alert** | All | 38 | 0.1 ± 1.4 | 0.1 ± 1.3 | 0.01 ± 1.70 | 38 | 0.4 ± 1.1 | 0.5 ± 1.1 | 0.08 ± 1.09 | 0.835 |
| | Optimal | 19 | 0.0 ± 1.3 | 0.0 ± 1.2 | 0.01 ± 2.04 | 19 | 0.6 ± 1.2 | 0.6 ± 1.2 | 0.08 ± 1.16 | 0.909 |
| | Sub-optimal | 19 | 0.3 ± 1.5 | 0.3 ± 1.3 | 0.01 ± 1.32 | 19 | 0.2 ± 2.0 | 0.3 ± 0.8 | 0.08 ± 1.06 | 0.820 |
| **Calm** | All | 38 | -1.5 ± 1.7 | -1.3 ± 2.0 | 0.22 ± 1.90 | 38 | -1.6 ± 2.3 | -1.2 ± 1.8 | 0.44 ± 2.52 | 0.664 |
| | Optimal | 19 | -1.3 ± 1.5 | -1.0 ± 2.2 | 0.34 ± 1.99 | 19 | -1.0 ± 2.1 | -0.6 ± 1.5 | 0.41 ± 2.46 | 0.903 |
| | Sub-optimal | 19 | -1.7 ± 1.9 | -1.6 ± 1.7 | 0.10 ± 1.85 | 19 | -2.2 ± 2.3 | -1.7 ± 1.8 | 0.47 ± 2.65 | 0.668 |
| **Content** | All | 38 | 0.3 ± 0.8 | 0.1 ± 1.1 | -0.26 ± 1.30 | 38 | 0.1 ± 0.8 | -0.1 ± 0.8 | -0.16 ± 1.01 | 0.701 |
| | Optimal | 19 | 0.3 ± 0.7 | -0.1 ± 1.2 | -0.42 ± 1.63 | 19 | -0.1 ± 0.7 | -0.3 ± 0.9 | -0.19 ± 1.08 | 0.602 |
| | Sub-optimal | 19 | 0.3 ± 0.8 | 0.2 ± 1.0 | -0.09 ± 0.89 | 19 | 0.3 ± 0.9 | 0.1 ± 0.6 | -0.12 ± 0.95 | 0.918 |
| **Fatigue** | All | 38 | 0.8 ± 1.8 | 0.4 ± 1.6 | -0.44 ± 2.30 | 38 | 0.3 ± 2.1 | 0.3 ± 1.7 | 0.02 ± 2.73 | 0.442 |
| | Optimal | 19 | 0.4 ± 1.2 | 0.0 ± 1.5 | -0.38 ± 2.23 | 19 | -0.3 ± 1.5 | 0.1 ± 1.7 | 0.39 ± 2.71 | 0.386 |
| | Sub-optimal | 19 | 1.3 ± 2.2 | 0.7 ± 1.7 | -0.51 ± 2.43 | 19 | 0.9 ± 2.4 | 0.6 ± 1.6 | -0.35 ± 2.77 | 0.847 |
| **Stress** | All | 38 | 0.8 ± 2.2 | 0.5 ± 1.9 | -0.27 ± 2.87 | 38 | 0.5 ± 1.9 | 0.3 ± 1.8 | -0.17 ± 2.51 | 0.884 |
| | Optimal | 19 | 0.0 ± 1.5 | 0.0 ± 1.7 | 0.02 ± 2.60 | 19 | 0.1 ± 1.7 | -0.2 ± 1.9 | -0.32 ± 2.86 | 0.740 |
| | Sub-optimal | 19 | 1.6 ± 2.5 | 1.1 ± 2.0 | -0.55 ± 2.43 | 19 | 0.8 ± 2.1 | 0.8 ± 1.6 | -0.02 ± 2.16 | 0.529 |
| **Reactivity** | All | 38 | 2.7 ± 5.2 | 2.0 ± 4.9 | -0.71 ± 5.92 | 38 | 1.4 ± 6.8 | 1.8 ± 5.2 | 0.40 ± 6.50 | 0.468 |
| | Optimal | 19 | 2.2 ± 4.7 | 1.2 ± 5.5 | -0.95 ± 7.09 | 19 | -1.1 ± 6.9 | 0.2 ± 5.2 | 1.26 ± 7.42 | 0.388 |
| | Sub-optimal | 19 | 3.3 ± 5.8 | 2.8 ± 4.3 | -0.47 ± 4.65 | 19 | 3.8 ± 6.0 | 3.4 ± 4.7 | -0.47 ± 5.50 | 1.000 |

Scores are presented as mean ± standard deviations. Δ indicates mean change score between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing the placebo to active intervention change scores. A higher score is indicative of a favourable neurocognitive result, whereas a lower score is indicative of a poorer neurocognitive result.

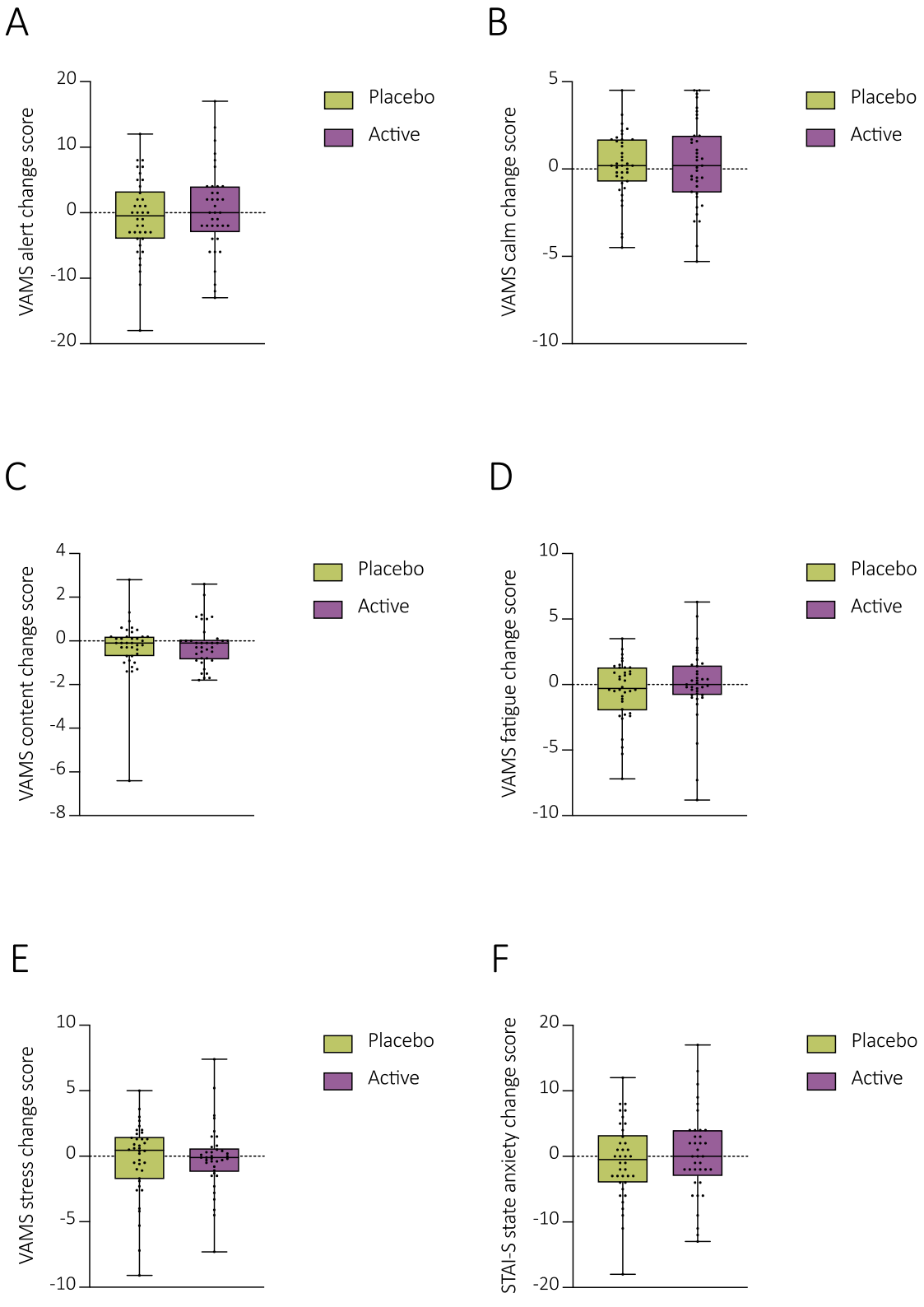



**Figure 3.4.** Effect of treatment on change in mood following cognitive stressor

Data presented for A) Alert, B) Calm, C) Content, D) Fatigue, E) Stress, and F) Reactivity

The VAMS and STAI-S change scores indicate the mean change scores between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing the placebo to active intervention change scores.

# Chapter 4: Discussion

## 4.1 Overview

Using a double-blind randomised placebo-controlled design, this thesis aimed to investigate the effect of a polyphenol-rich blackcurrant beverage on neurocognitive outcomes among healthy females. The current study assessed changes in stress reactivity to a multi-tasking cognitive stressor, including acute changes to subjective anxiety, mood, and stress, and cognitive performance including attention, psychomotor function, working memory, and an aggregate multi-tasking score. This chapter begins with a summary of the main findings, followed by an interpretation of these findings within the context of the existing literature. It is important to note, this chapter primarily focuses on existing long-term polyphenol supplementation studies, although acute polyphenols supplementation studies are also examined when discussing these study findings. The strengths and limitations are then explored and lastly, the directions for future research are presented.

## 4.2 Summary of key findings

To provide some context to the discussion of these findings, the LINK study cohort was a generally well-educated, healthy population, with the majority of the cohort meeting recommended nutrient intakes (**Table 3.1**) (132). As a reminder, participants were recruited with a balance of 'optimal' and 'sub-optimal' diets according to the DST, aiming to capture a wider distribution of diet quality. Contrasting with the only other study to utilize this recruitment strategy (2), the current study found no significant differences in macronutrient or micronutrient intakes between the 'optimal' and 'sub-optimal' dietary groups according to data analyzed from three-day food records. The DST extends beyond mere nutrient intake, serving as an indicator of diet quality and variety- a facet perhaps not captured when considering nutrient intakes alone.

The key finding of this study was improvements in letter search scores and a trend towards improved total scores of the Purple-MTF following daily supplementation with the Ārepa beverage for four weeks compared to placebo, indicating improvements in working memory and executive function. Interestingly, we found the 'sub-optimal' diet group demonstrated significant improvement in letter search and total scores of the Purple-MTF following supplementation, however the same response

was not seen in the 'optimal' diet group. Despite improvements to cognitive function, we did not find any difference in changes to stress reactivity measures (alertness, calmness, contentment, fatigue, and stress) between active and placebo interventions in the total study population, or in analyses separating 'sub-optimal' and 'optimal' diet groups.

## 4.3 Comparison to existing literature

### 4.3.1 Cognition

The current study is the second to examine the neurocognitive impact of the Ārepa nootropic beverage. The first study investigated the effect of Ārepa on cognitive function in a sport setting (3), also finding improvements in cognitive function following supplementation (107). The double-blind, placebo-controlled, cross-over RCT specifically explored the effect of daily Ārepa consumption on cognitive performance among sub-elite rugby league players following a one-week supplementation period (107). Following a standardized training session to induce cognitive fatigue, Ārepa was found to positively impact cognitive performance, significantly improving the Stroop test total score, accuracy, and average time per response (107). The current study reported in this thesis also involved a Stroop test, however daily Ārepa supplementation was found to have no improvement in the total Stroop score in the context of the multi-tasking framework. Although the current study did not measure Stroop accuracy or average time per response, we found other measures of cognitive function improved, including executive function, and working memory.

The Ārepa beverage contains a unique blend of blackcurrant, L-theanine, and Enzogenol® extracts. Given the limited comparable literature on the unique Ārepa formula, it is useful to examine the existing literature according to each constituent bioactive. A variety of studies have explored the effect of daily anthocyanin-rich fruits, L-theanine, or Enzogenol® supplementation on neurocognition among healthy populations (57,90,92,103,105,109–112,129,136,137). For example, one study investigating the effect of daily blackcurrant supplementation found no benefit on cognitive function, which contrasts with the findings of this thesis. The double-blind, placebo-controlled, cross-over study explored the effect of a New Zealand blackcurrant extract, providing 210mg of anthocyanins per day (a similar dose of anthocyanins to the current study), on cognitive function among older adults (n=14) following a one-week supplementation period (110). Daily blackcurrant supplementation had no effect on cognitive function variables according to the Cambridge Neuropsychological Test.

Automated Battery (CANTAB), including reaction time, paired associates learning, spatial working memory, and rapid visual processing (110). While these findings contrast those of the present study, there are several potential reasons driving the discrepancies. For example, the intervention beverage of the current study had a notably higher dose of anthocyanins (311mg per day) than the comparative study (210mg per day), and also contained an additional source of flavonoids from the 150mg of Enzogenol®. Although testing similar domains of cognition, the CANTAB and Purple-MTF cognitive tests are of course not directly comparable which may also contribute to the discrepancies in findings.

Although there are limited studies investigating daily blackcurrant-based supplementation and neurocognition, we can draw on the more extensive literature available for the other anthocyanin-rich fruits, beverages, and supplements. There are a modest number of robust studies investigating daily supplementation of other anthocyanin-rich ingredients (blueberry, black chokeberry, cherry, grape juice, and grape seed extract) on cognitive function (57,90,92,103,105,111,112). These studies with long-term treatment periods (ranging from three to six months), particularly those with daily anthocyanin doses of >195mg have trended towards an improvement in cognitive function. Long-term studies have shown the most notable effect of polyphenol supplementation on attention function, episodic memory, and working memory domains of cognitive function (57,92,103,105,112). In addition to long-term supplementation, studies involving acute polyphenol supplementation among healthy participants have also demonstrated neurocognitive benefits (27). In particular, acute polyphenol supplementation has shown to support memory and attentional aspects of executive function (93,94,98,100–102).

Considering the limited and inconsistent findings on the effect of long-term L-theanine supplementation on cognitive function, drawing conclusions in the context of this study is challenging. The two studies investigating the effect of long-term L-theanine supplementation on cognitive function among healthy populations have reported conflicting findings (129,136). Similar to our own findings, one study demonstrated that four weeks of the same 200mg daily dose of L-theanine supplementation significantly improved cognitive function, including verbal fluency and executive function scores (129). In contrast, another study involving a notably lower daily dose of L-theanine (100.6mg) found that 12 weeks of supplementation had no effect on cognitive performance (136). Compared to long-term supplementation, studies involving acute L-theanine supplementation among healthy participants have demonstrated a more consistent cognitive benefit. In particular, acute L-theanine supplementation has identified a positive effect on memory and attention function (136,138,139).

Similar to findings of the current study, the only known study to investigate the effect of daily Enzogenol® supplementation on neurocognition also found an improvement in cognitive function. Compared to the active vitamin C control, five weeks of daily Enzogenol® supplementation (960mg) plus vitamin C produced a beneficial effect on cognition among healthy males, including improving the speed of response on the immediate recognition and spatial working memory tasks (109). There are no known studies exploring the effect of acute Enzogenol® supplementation on neurocognition. Considering the limited research examining Enzogenol® supplementation and cognitive function, the potential effect in the context of this study is largely unknown.

In summary, the findings from the current study align with existing literature, where varying domains of cognition are positively impacted by anthocyanin, L-theanine, and Enzogenol® supplementation. There is more robust existing research on anthocyanins compared to other constituent ingredients, and findings from the current study are generally congruent with this literature which demonstrates the potential of anthocyanins in supporting specific domains of cognition, including executive function, working memory, and attention function. This is in accordance with potential mechanisms in which polyphenols provide neurocognitive benefits, particularly through enhancement of cerebral blood flow and connectivity in the hippocampus, while also decreasing neuroinflammation and oxidative stress (140,141) These beneficial effects are specifically linked to the cognitive processes in memory and frontal executive function (60).

In the context of scarce research on the unique Ārepa formula, it should be highlighted that direct comparisons to constituent ingredients are limited by the fact that the three bioactive ingredients plausibly have additive or synergistic effects on neurocognitive function. It is also important to note that study methodology differs considerably across the literature, making comparisons across studies challenging in light of heterogeneity in polyphenolic dosage, polyphenol treatment, intervention duration, neurocognitive assessment, age group, population size, and washout period.

### 4.3.2 Stress reactivity

There is limited comparable literature on the unique Ārepa formula when considering measures of stress reactivity, and examining the existing literature according to each constituent bioactive is again necessary. A small number of studies have explored the effect of daily anthocyanin-rich fruits and L-theanine supplementation on stress reactivity among healthy populations (107,112,129,137). However, there are no studies examining daily Enzogenol® supplementation and stress reactivity, and therefore the potential effect in the context of this study is unknown.

Although not directly comparable to the stress reactivity measures used in this thesis before and after a cognitive stressor, it is interesting to briefly note that previous research on the commercially available Ārepa nootropic beverage has examined intervention effects on mental clarity in a sports setting (107). In this study, participants completed a modified, validated mental toughness questionnaire (MTQ48) at three intervals during the one-week supplementation period, revealing improvements in perceived reliability and reduced perceived distractibility (107).

Unlike the findings from the current study, the only known previous study to investigate the effect of daily polyphenol supplementation on stress reactivity supported some improvements to components of stress reactivity following supplementation (112). This study examined the effect of tart cherry on stress reactivity and found the polyphenol-rich supplement to improve perceived alertness and mental fatigue but did not improve perceived calmness or contentment according to the Bond-Lader visual analogue scale (112). Although involving similar stress reactivity questionnaires, the current study revealed contrasting findings, with no improvements in perceptions of alertness or mental fatigue following supplementation with the polyphenol-rich beverage, however we corroborate the lack of response regarding perception of calmness and contentment.

Again, contrasting with the current study's findings, research investigating the effect of L-theanine supplementation has demonstrated a positive effect on stress reactivity. There is a gap in the literature regarding the effect of daily L-theanine supplementation on stress reactivity. However, two separate double-blind, cross-over RCTs investigated acute supplementation of 200mg of L-theanine on stress reactivity following cognitive stressors (127,142). Unlike the current study, both studies found L-theanine supplementation demonstrated a positive effect on stress reactivity according to VAMS and STAI questionnaires (127,142). While there is a paucity of research on L-theanine supplementation and stress reactivity, other studies have included the effect of L-theanine

supplementation on stress biomarkers (127,137,142). Acute 200mg L-theanine supplementation has shown to decrease heart rate, salivary cortisol, salivary immunoglobulin A, and subjective baseline stress (127,142,143). The observed positive effects on the stress response were proposed to be an attenuation of sympathetic nervous activation (142). L-theanine has also shown to reduce baseline stress prior to cognitive stressors (137,143). In the current study, an analysis of baseline subjective stress was not conducted due to the increasing risk of type I errors.

In summary, the findings from the current study do not align with the existing literature, whereby polyphenol and L-theanine supplementation have been shown to positively impact measures of stress reactivity albeit from a limited number of studies (13,14). Although Ārepa supplementation was shown not to positively support stress reactivity, there is a possibility that baseline subjective stress could have been improved, however that measure was not analysed in the current study. The lack of an observed positive effect on stress reactivity may also be related to the cognitive stressor itself, which differs from the cognitively demanding tools designed to elicit mental fatigue in previous research on polyphenols (112). Although previous research has shown that the Purple-MTF is capable of eliciting changes to stress, alertness, and calmness (123), it may not have been sufficient to detect intervention effects amongst our cohort of healthy females.

## 4.4 Strengths and limitations

The present study was a novel exploration of Ārepa, and its unique combination of ingredients and neurocognitive function among a sample of healthy females. Adding a valuable perspective, the present study contributes to existing literature of nootropic beverages and neurocognition. The rigorous methodological design was a major strength of the present study. The study was a double-blind, placebo-controlled, cross-over RCT, including a wash-out period between intervention arms. The current study's cross-over design can be considered as a notable strength when compared to existing literature, as the majority of studies assessing the effect of daily polyphenol supplementation on neurocognition among healthy populations have not involved a cross-over design which enables participants to act as their own control (57,90,103,105,111,112). Despite potential questions around whether the Purple-MTF was sufficiently sensitive to detect intervention effects in the current research, another methodological strength to note is that compared to other cognitive stressors such as simulated public speaking, the Purple-MTF can be repeated by the same participant on several occasions with minimal learning effects (124) which makes it suitable for use in cross-over trials (125). Another strength included the observed improvements to cognitive function in a research paradigm that reflects day-to-day life stress.

Another strength included our novel approach to participant recruitment. Self-selection bias is a pervasive issue during recruitment in dietary intervention trials (113). This traditional sampling method characteristically attracts health-conscious participants who are likely to have optimal nutrient status prior to the intervention (113). Our study illustrates this recruitment phenomenon, whereby we turned away 26 participants because the 'optimal' diet group was already satisfied (Figure 3.1). Research continues to employ flawed convenience sampling, including neglecting baseline diet and nutrient status and a disregard of its potential impact on the integrity of the findings (144). Self-selection bias has the potential to considerably undermine any treatment effects, therefore increasing the risk of type II error among the field of nutritional science (113).

Although the novel screening method utilised in the current study was time-consuming, it enabled the identification of subgroups most likely to benefit from the treatment. Categorizing participants into 'optimal' and 'sub-optimal' diet groups allowed the analysis of differential supplement effects according to baseline diet quality. We identified a significant benefit of daily Ārepa supplementation for cognitive function in the 'sub-optimal' diet group, with the same response not found in the 'optimal' diet group. If the current study had not excluded those additional 26 participants with an already optimal diet during recruitment, we would have risked masking the effects of the treatment beverage. This study builds on the emerging body of evidence demonstrating the importance of baseline participant profiling, calling for the use of a priori dietary screening in future dietary intervention studies.

Although the study provides some unique insights, it is also important to consider its limitations. Firstly, the intervention treatment was a pre-existing, commercially available nootropic beverage. As the Ārepa nootropic beverage contains multiple bioactive ingredients, including blackcurrant, L-theanine, and Enzogenol®, this limits the generalisability of the findings. Although blackcurrant is the main component, the results observed cannot be solely attributed to this ingredient. The supporting ingredients may have also contributed to the cognitive outcomes observed. Given the scarce research available on the unique Ārepa formula, it is important to recognise that direct comparisons to individual ingredients was limited, with the three bioactive ingredients likely to exert additive or synergistic effects on cognitive function. Another factor affecting the generalisability of the findings was the study cohort. As the study population was healthy females, the findings of the study therefore cannot be extrapolated to other populations, including males and older adults.

The current study also had limitations in drawing inferences about the potential underlying mechanisms responsible for the observed neurocognitive benefit. Although potential mechanisms are being investigated in the broader LINK study including BDNF and gut microbiota composition, these biological markers were beyond the scope of the current thesis. Other considerations to aid in exploring potential mechanistic actions, such as measure of plasma anthocyanin content, and cerebral blood flow using near-infrared spectroscopy and magnetic resonance imaging were not utilised in the LINK study which does limit the study's ability to offer insight into the factors driving the observed neurocognitive benefits from this thesis.

## 4.5 Future directions

This thesis has created exciting opportunities for future research, addressing some of the limitations raised in the prior section. Firstly, future high-quality RCTs are still warranted to further explore the potential of long-term polyphenol supplementation on neurocognition. In particular, long-term polyphenol supplementation studies should involve testing among various neurological and psychological disease states. Polyphenol supplementation has significant potential to attenuate age-related cognitive decline, the development of neurological disorders, including dementia, and even protect neurocognition function among patients with traumatic brain injury (141,145). Clinical trials have begun to demonstrate the potential of daily polyphenol supplementation in supporting neurocognition among a range of neurological and psychological disease states, including mild cognitive impairment, depression, and dementia (59,88,104,108,116,146). This preliminary research requires well-designed long-term prospective clinical trials to substantiate the efficacy of polyphenol supplementation in attenuating cognitive impairment and the development of neurological disorders. To enhance the existing literature, future research should incorporate cross-over designs, employ larger sample sizes, and extend intervention and follow-up periods. Both acute and long-term dose-response studies are also required, assessing multiple polyphenol doses among the same population to better understand optimal dosing recommendations (67).

The exact mechanisms underlying the neurocognitive benefit of polyphenols are complex and poorly understood (147,148). Further investigation is required to identify the mechanisms that link polyphenol supplementation with improved cognition (147). Imaging and spectroscopic techniques such as functional magnetic resonance imaging and nuclear magnetic resonance should be utilized in future studies (27,141). These techniques will be important in understanding the mechanistic

polyphenol-based changes in cerebral blood flow, neuronal stem cells, and grey matter (141). Future in vivo studies should further examine these mechanistic actions, assisting in the development of guidelines for polyphenol supplementation and clinical recommendations in various neurological and psychological disease states (141). Recommendations should include optimal dose, duration, bioavailability, and composition of polyphenol treatment for safe and efficacious results, all of which are currently unknown but urgently needed to harness their potential (148).

Innovative delivery mechanisms have been proposed to enhance anthocyanin absorption and effect on neurocognition (47,148,149). Nanoparticle targeted drug delivery is a valuable emerging tool in neurotherapeutics due to its ability to penetrate the blood-brain barrier and improve the delivery of drugs to the brain (47). The neuroprotective potential of anthocyanins has been explored using anthocyanin-loaded poly (ethylene glycol)- gold nanoparticles (PEG-AuNPs) in amyloid-beta ($A\beta_{1\text{-}}42$) injected into in vitro Alzheimer's disease models (47). Compared to anthocyanins alone, anthocyanins coupled with PEG-AuNPs displayed greater effectiveness in decreasing $A\beta_{1\text{-}42}$-induced neuroinflammation and neuro-apoptosis markers in vitro Alzheimer's disease models (47). Anthocyanins coupled with PEG-AuNPs can transport across the BBB, demonstrating neuroprotective benefits and acting as a potential therapeutic agent for neurodegenerative diseases (39). Future studies should explore the potential of anthocyanin-loaded PEG-AuNPs as a therapeutic agent for various age-associated neurodegenerative diseases, including Alzheimer's disease.

## 4.6 Conclusions

Using a cross-over, double-blind randomised control design, the LINK study investigated the effect of Ārepa, a polyphenol-rich blackcurrant drink, on neurocognitive outcomes, including stress reactivity and cognitive function. The findings of the current study generally align with the literature, adding to the body of evidence that demonstrate the benefit of polyphenols in supporting specific domains of cognition, including executive function, working memory, and attention function. The key finding of this study was improvements in the letter search and total scores of the Purple-MTF following daily supplementation with the Ārepa beverage for four weeks compared to placebo, indicating improvements in working memory and executive function. When considering the effects of baseline diet quality on neurocognitive responses, the 'sub-optimal' diet group demonstrated significant improvement in letter search and total scores of the Purple-MTF following supplementation, however the same response was not observed in the 'optimal' diet group. Despite improvements to cognitive

function, daily Ārepa supplementation had no positive effect on any stress reactivity measures. Future high-quality RCTs are required to strengthen the evidence-base, particularly exploring long-term polyphenol supplementation among various neurological and psychological disease states. These studies should examine mechanistic actions using imaging and spectroscopic techniques to inform recommendations, including optimal dose, duration, bioavailability, and composition of polyphenol treatment for safe and efficacious results.

# Appendices

1. Participant information sheet

2. Participant consent form

3. Dietary Screening Tool

4. Questionnaires

    a. State Trait Anxiety Inventory: State Subscale (STAI-S)
    b. Visual Analogue Mood Scale (VAMS)

5. Effect of treatment on change in MTF maths, Stroop, and tracking scores

## Appendix 1. Participant information sheet

MEDICAL AND HEALTH SCIENCES

### PARTICIPANT INFORMATION SHEET

### Polyphenol-rich drink for gut and brain health (LINK Study)

#### Invitation

You are invited to take part in this study which aims to understand the effects of a blackcurrant-based beverage on markers of the gut-brain axis. As a volunteer, it is important for you to understand why we are doing this research, and to understand what will be involved if you decide to participate.

This participant information sheet will help you decide if you would like to take part. It describes why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. **Please take time to read this information sheet carefully, and contact us if anything is not clear or you would like further information. Please also talk to other people like family/whānau, friends, or healthcare providers to help you decide whether you would like to take part in the study**. You will have the opportunity discuss the information presented here with the study team, who can answer any questions you might have.

If you agree to take part in this study, you will be asked to find the Consent Form attached at the end of this document. You will be given a copy of this information sheet and the signed consent forms.

#### What is the LINK study?

The gut microbiome (gut bacteria) plays an important role in many aspects of our health, particularly our mood and mental wellbeing. We are carrying out the LINK study to find out whether a blackcurrant-based beverage (Ārepa) can provide benefits to markers of the gut-brain axis, including the gut microbiome, neurocognitive responses (cognition, mood, sleep), and related markers in the blood. We will also explore whether baseline diet or the gut microbiome mediates the effects of the blackcurrant drink on study outcomes.

The LINK study will recruit 40 healthy female adults to participate in a 3-month long intervention. The study period includes four weeks on one arm of the intervention (Ārepa performance beverage or placebo beverage), four weeks of a 'wash-out' period, and then four weeks on the second arm of the intervention. The order of intervention will be randomised, and there will be 5 study visits in total. The first will be a screening visit and take approximately 1-hour, the following four will be study visits conducted in the morning which take approximately 45-minutes. We will be collecting blood and stool (poo) samples, and you will also complete questionnaires and cognitive tasks during the study.

#### What is the purpose of the LINK study?

There is an increasing number of people with functional gastrointestinal disorders, like irritable bowel syndrome. These conditions are thought to be driven by altered communication along the gut-brain axis. Strategies which improve microbiota-gut-brain interactions are thought to be important in optimising gastrointestinal health and preventing the onset of functional gastrointestinal disorders. Translational to practical solutions are still needed though.

Several dietary components might have positive or negative impacts on aspects of microbiota-gut-brain interactions. The New Zealand blackcurrant cultivar Black Adder is rich in anthocyanins, a class of plant-based compounds called polyphenols. Previous data in animals and humans has shown a prebiotic effect of anthocyanins, leading to the growth of health-promoting bacterial species in the gut. Supplementation with the Black Adder cultivar has also been shown to favourably impact brain physiology and behaviour. Integrative studies which simultaneously measure the microbiota, neurocognitive responses, and related blood biomarkers are needed to better understand the potential of polyphenols.

The Ārepa beverage is rich in anthocyanins and other plant-based ingredients known to provide neurocognitive benefits (L-theanine, pine-bark extract). We hypothesize that this unique formula will enhance microbiota-gut-brain interactions through dual effects on the gut microbiota and neurocognitive responses.

## Who are the researchers?

This study involves a team of researchers from the University of Auckland (Dr Nicola Gillies, Dr Tommi Vatanen, Dr Andrea Braakhuis), the University of Otago (Prof Nicole Roy) and Swinburne University of Technology (Prof Andrew Scholey). These are the researchers who have designed this study. Dr Nicola Gillies is the Principal Investigator and will be managing the study. The research team includes registered dietitians, neuroscientists, and specialists in research on the gut microbiota.

## Who is funding the study?

The LINK study is funded by grants from the Ministry of Business and Innovation (MBIE) and AlphaGen Ltd through the National Science Challenges – High Value Nutrition (www.highvaluenutrition.co.nz).

## Who can participate in the LINK study?

This study may be suitable for you if you are a healthy female, and aged between 18-45 years at the time of enrolment.

We cannot include people who have taken antibiotics in the four weeks prior to the study starting, or who are diagnosed with gastrointestinal disorders (e.g., Coeliac Disease, Inflammatory Bowel Disease), who have been treated for anxiety, depression or psychiatric disorders within the past two years, or who have a history of neurological disorders (e.g., Epilepsy, serious head trauma) or cognitive impairment. This is because these conditions might impact our study outcomes.

If you are taking prebiotic, probiotic or other supplements which might impact our study outcomes we ask that you stop taking these four weeks prior to the study starting and for the duration of the study period. You can ask us if you're sure about which supplements might be relevant here.

No one has to take part in the LINK study, and it is completely up to you to decide whether or not to take part in the study. If you do decide to enrol in the study, you can withdraw at any time without giving a reason.

## What will my participation in the LINK study involve?

**Online screening:** If you are interested in participating, you will complete an online screening questionnaire to make sure that you meet the criteria for taking part. If you are eligible according to this questionnaire, you will be invited to attend a screening visit at the Grafton Clinical Research Centre (University of Auckland).

**Screening visit:** If you agree to attend this study visit, researchers will review this participant information sheet with you in person, explaining the study in detail and answering any questions you may ask about participation. If you are satisfied and agree to take part, we will ask you to sign the consent form which can be found at the end of this document. At this point you will be officially enrolled into the LINK study.

After enrolment, you will be familiarised with the computerised multi-tasking programme we use in the study to trigger cognitive stress. You will also complete some questionnaires at this visit, which are repeated online during the study. These questionnaires will give us information on your mood, sleep quality, dietary intake, and physical activity. At the end, you will be given instructions and materials for stool (poo) sample collection which allows us to measure your gut bacteria. This screening visit will take around 1 hour in total.

**Study period:** Approximately two weeks after your first study visit you will start the study, which takes three months in total. This involves four weeks on the first study beverage ("arm 1") where you will consume one 300mL beverage daily, four weeks in a 'wash-out' period where you do not consume any study beverage, and then four weeks on the second study beverage ("arm 2") where will you will consume the other 300mL beverage daily. You will be supplied with all the beverages needed for your participation in this study at no cost.

This trial is 'blinded', which means that neither you nor the researchers will know whether you are having the study treatment (Ārepa) or the taste- and colour-matched placebo beverage. This information is only known to someone unconnected with the trial. The order in which you consume the study beverages will be randomly assigned after you are enrolled in the study. This study design helps to make sure that the researchers interpret the results in a fair and appropriate way, and avoids researchers or participants jumping to conclusions.

Both beverages are manufactured in a registered facility that complies with Food Standards Australia and New Zealand guidelines, with respect to manufacturing standards and compliance with food safety requirements.

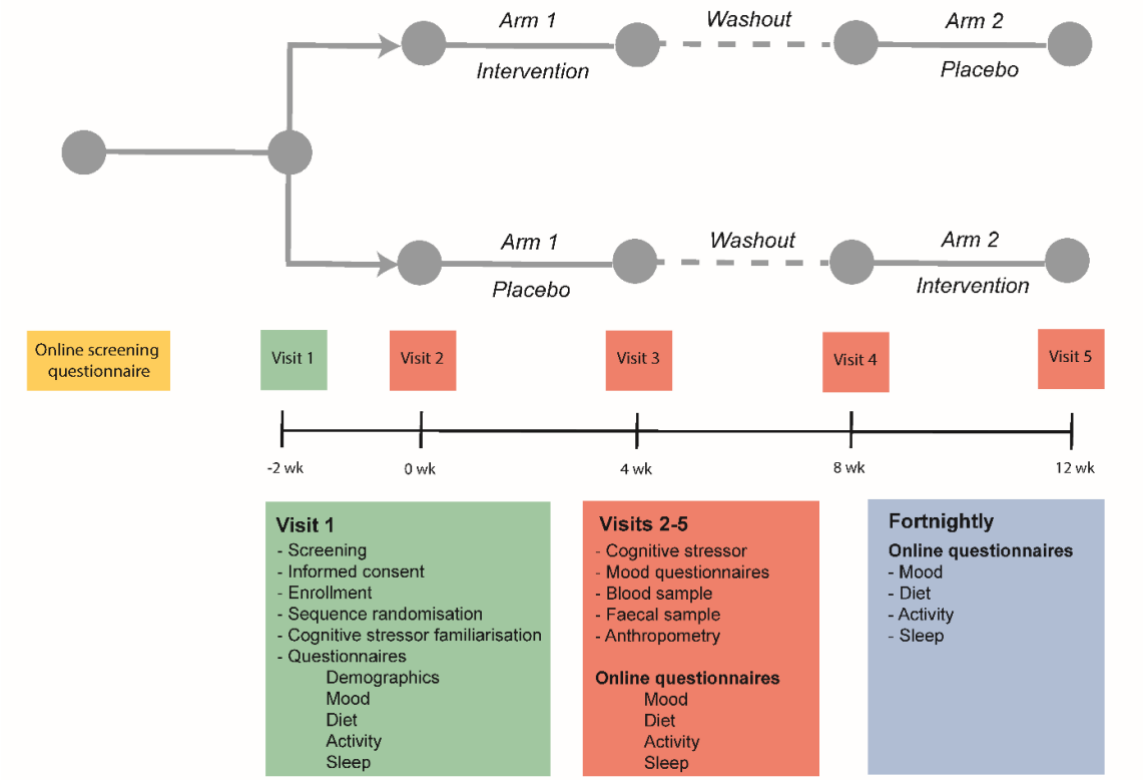
**Data collection**: There are 4 visits during the study period, where data will be collected in person. These happen at the start and end of each intervention arm (i.e., every four weeks), and are expected to take around 45 minutes each. Each study visit will have similar testing procedures. All data will be collected at the Clinical Research Centre in Grafton, Auckland. You will be asked to avoid alcohol the day before your study visit, caffeine in the 12h prior to your appointment, and to not eat after 10pm. Except for water, you will fast overnight and attend your scheduled study visit in the morning.

You will provide researchers with a stool (poo) sample which was collected at home, in the 24h before your study visit using the kit and instructions provided at previous study visits.

Height and weight will be taken, and blood samples will then be collected. You will be asked to rest on a bed or chair, and a small needle will be placed into your arm vain. This can be slightly painful, and can cause discomfort. The researcher will then take approximately 15mL of blood which will be used to measure inflammatory markers (circulating cytokines), neurocognitive markers (monoamine oxidase B, brain derived neurotropic factor), and amino acids and their metabolites (serum tryptophan, kynurenine).

You will then complete questionnaires which evaluate your mood in the present moment. After this, you will complete the cognitive stressor which takes approximately 20 minutes. Finally, you will repeat the questionnaires on your mood. This allows us to measure your mood under conditions of stress or no stress.

You will complete online questionnaires at fortnightly intervals during the study which provide information on your diet, mood, sleep, and physical activity. These will take approximately 15 minutes of your time or less. At weekly intervals you will need to confirm that you have consumed the study beverage through a quick online questionnaire.



## What will happen to my blood and stool samples?

**Blood samples:** Your blood will be used to analyse inflammatory markers, neurocognitive markers, and amino acids and their metabolites. These markers are related to the gut and/or brain and provide insights into whether there are differences in the response to the intervention or placebo beverage, and how the intervention might be achieving these outcomes.

Blood samples will be collected, prepared, and stored at the Nutrition Department Laboratory (Faculty of Medical and Health Sciences, University of Auckland). Analysis of blood samples will take place at the Liggins Institute Laboratory (University of Auckland) or Plant and Food Research (Palmerston North, New Zealand). After these analyses have been performed, it will not be possible to return any unused samples to you. You can request the return of your blood prior to any analysis; this would mean we would not use your information in the study.

**Stool samples:** We will measure changes in gut microbiota (through identifying and classifying the types of gut bacteria present), microbiota diversity, and the predictive function of your gut bacteria (through measuring the genetic material of the gut bacteria) from your stool samples. These samples will be processed at the University of Auckland. A small sample will be sent to an overseas laboratory (Beijing, China), frozen on dry ice, for expert analysis of gut bacteria that cannot take place in New Zealand.

**Transport and storage:** All blood and stool samples will be transported locally, nationally, and internationally according to international guidelines for the transport of human tissue. All samples will be labelled with your LINK study ID number and not your name, to maintain confidentiality.

Iwi, hapu, and whānau might disagree with transport of tissue samples due to issues with the loss of rights to your whakapapa. It is acknowledged that individuals have the right to choose, and these concerns might also apply to non-Māori. We encourage you to consult with your whānau before agreeing to participate. You can also have a support person contact us or attend your screening visit if you have any questions.

All samples will be labelled with your LINK study ID and will be stored in secure freezers in an access-restricted area at the University of Auckland until analysis. Any unused samples collected in this study will be kept for a total of 10 years. At the end of this time, a medical waste contractor will dispose of your tissue. If you would like a karakia said at this time, please indicate so in the consent portion of this form. Any samples for disposal by karakia will be clearly marked. It is possible that the entire sample will be used for analysis, in that case there will be no need for disposal and a karakia will not be possible. Karakia ceremonies take place through the Auckland District Health Board. Stored tissue will not be used for any future unspecified research purposes.

**Detection of abnormalities:** We will advise you of any abnormal test results found as part of the study which might have implications for your future health, including results from questionnaires, blood samples, and stool samples. These findings will be provided to you, along with a letter for your doctor. We will contact your doctor or an appropriate specialist if you agree.

If you do not wish to be informed of any results indicating a possible medical concern, you cannot participate in this study.

## What are the benefits or risks of taking part in the LINK study?

**Risks:** There are no major risks associated with taking part in this study. You do need to be aware that this study will involve collection of a blood sample. This will be performed by an experienced researcher. A blood sample may hurt a little, and some people get a small bruise where the needle goes in. Occasionally, this can become infected but this is very rare and most people have no problems. If you ever faint with blood samples or when you see blood, please let the researchers know beforehand. That way, we can be prepared for this and take the sample while you are lying down.

There is minimal risk in other procedures associated with the trial, but acknowledge that the multi-tasking activity and questionnaires can bring about disturbances in mood which people might find uncomfortable. Psychological assessments will be checked weekly by the research team. If any questionnaires raise concerns, these will be reviewed by a neuroscientist in the research team, who may suggest referral to counselling services if needed with your permission.

Researchers will check in on your safety regularly through the trial. Any adverse events (e.g. reaction to the beverages such as gastrointestinal upset) or serious adverse events that emerge or worsen relative to your usual state will be recorded and reported to the Health and Disability Ethics Committee.

**Benefits:** There are many benefits to being involved in this research. You will have several assessments which are not usually available through standard care, receive information about how you respond to an experimental stressor, and receive information about your gut microbiota. You will be acknowledged in publications (anonymously), and be provided with the study findings. Your involvement in this study is of great value to the researchers and will help to advance understanding on how dietary components affect the gut-brain axis, thank you for considering taking part.

## How will my confidentiality be protected?

Your confidentiality and protection of personal information will be treated very seriously, and individual results from this study will be kept strictly confidential. On entering the study, you will be given a unique study identification number/ID, which will be used on all forms, questionnaires, measurements, and blood/stool samples. Researchers will remove any personal information provided by you, and there is no risk that you will be able to be identified if samples are sent nationally or overseas. It is important to note that privacy protections in other countries may be different to those offered in New Zealand, but using your study ID will protect your identity. Researchers will analyse the whole study group's data and report on averages in any scientific publications and presentations and no person will be identifiable.

Any hard-copy documents, including paper copies of questionnaires, data collection forms or measurements will be stored in a locked filing cabinet in a secure swipe-access area at the University of Auckland, where only the research team has access. Any information on electronic files will only be accessible by the research team. The LINK study researchers responsible for data collection and management have been trained by an independent committee at the Greenlane Coordinating Centre (GLCC), who ensure the study is carried out according to guidelines for Good Clinical Practice.

## What happens if I suffer harm, injury, or complications because of the study?

It is unlikely that you will suffer any harm or complications because of this study. If you were injured as a result of treatment given as part of this study, you won't be eligible for compensation from ACC. However, Dr Nicola Gillies has satisfied the XXX Health and Disability Ethics Committee that approved this study that it has up-to-date insurance for providing participants with compensation if they are injured as a result of taking part in the study.

New Zealand ethical standards require compensation for injury to be at least ACC equivalent. Compensation should be appropriate to the nature, severity and persistence of your injury and should be no less than would be awarded for similar injuries by New Zealand's ACC scheme. Some sponsors voluntarily commit to providing compensation in accordance with guidelines that they have agreed between themselves, called the Medicines New Zealand Guidelines (Industry Guidelines). These are often referred to for information on compensation for commercial clinical trials. There are some important points to know about the Industry Guidelines:

- On their own they are not legally enforceable and may not provide ACC equivalent compensation.
- There are limitations on when compensation is available, for example compensation may be available for more serious, enduring injuries, and not for temporary pain or discomfort or less serious or curable complaints.
- Unlike ACC, the guidelines do not provide compensation on a no-fault basis. The Sponsor may not accept the compensation claim if:
    - Your injury was caused by the investigators, or;
    - There was a deviation from the proposed research plan, or;
    - Your injury was caused solely by you.

An initial decision whether to compensate you would be made the by the sponsor and/or its insurers. If they decide not to compensate you, you may be able to take action through the Courts but it could be expensive and lengthy, and you might require legal representation. You would need to be able to show that your injury was caused by participation in the trial. You are strongly advised to read the Industry Guidelines and ask questions if you are unsure about what they mean for you.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

You may have your friend, family, or whānau support help you understand the risks and/or benefits of this study or any other explanation you require. You are also welcome to have a friend or whānau support with you during every session.

## What will happen to my information?

All data will be stored electronically in password-protected files on a secure network drive managed by the University of Auckland. With your consent, your study records will be stored securely for 10 years after the study is completed and then destroyed. Your blood and stool samples will also be stored for 10 years after the study ends, which will be stored securely in the Nutrition Department freezers at the University of Auckland. If you decide to withdraw from the study, you may request that your samples are disposed of.

## What happens with the results of the study?

If you give us your permission by signing the Consent Form, findings from the study will be used in internal reports, scientific/professional conference presentations, and scientific journal. The findings may also be featured in the media. You will not be identified in any presentations or publications.

At the end of the study, we will provide you with a summary of results from the study. Please note that there may be a delay between your study visit and publication of the results.

## What happens if I change my mind?

You have the right to withdraw from the study at any time. Your contribution is entirely voluntary. If you decide to withdraw from the study, data that has already been obtained may be kept and used to contribute to the overall results. However, you can request that any data or information relating to you can be destroyed and we will ensure that this happens.

## Will taking part in the LINK study cost me anything, and will I be reimbursed?

You will not incur any costs for taking part in this study except for your time, for which we thank you. All study treatments and data collection will be paid for by the study funders.

We appreciate that taking part in this study involves 5 visits to our research centre, and approximately 4-5h of your time. All participants will receive a koha (gift) in the form of vouchers as an expression of thanks for dedicating time to this research. Vouchers will be provided at completion of each arm of the intervention (study visits 3 and 5). When visiting the Grafton Campus for research visits reserved parking will be arranged for you by the study team. Please let the study team know if there is a problem getting to your appointment, as arrangements can be made.

MEDICAL AND HEALTH SCIENCES

## Who do I contact for more information or if I have concerns?

Once you have read this information, a member of the study team will discuss it with you and answer any questions you may have.

If you have any questions, concerns, or complaints about the study at any stage you can contact:

Dr Nicola Gillies, Principal Investigator
Discipline of Nutrition,
Faculty of Medical and Health Sciences
The University of Auckland, New Zealand
Email: n.gillies@auckland.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz
Website: https://ww.advocacy.org.nz/

For Māori cultural support, talk to your whānau in the first instance. Alternatively, you may contact the administrator for He Kamaka Waiora (Māori Health Team) by telephoning 09 486 8324 ext 2324

You may also contact the health and disability ethics committee (HDEC) that has approved this study:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz

**APPROVED BY THE HEALTHY AND DISABILITY ETHICS COMMITTEE ON 27/05/2022, Reference Number (2022 EXP 12513)**

MEDICAL AND HEALTH SCIENCES

# CONSENT FORM

## THIS FORM WILL BE HELD FOR A PERIOD OF 10 YEARS

## Polyphenol-rich drink for gut and brain (LINK Study)

Principal Investigator: Dr Nicola Gillies
Co-Investigators: Dr Tommi Vatanen, Prof Nicole Roy, Prof Andrew Scholey, Dr Andrea Braakhuis

*The investigators conducting this research abide by the principles governing the ethical conduct of research as set out by the World Medical Association, Declaration of Helsinki (2008) and the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007) and at all times avow to protect the interests, comfort, and safety of all subjects. This form and the accompanying participant information sheet have been given to you for your own protection.*

**As a participant, please tick to indicate consent to the following;**

| | |
|---|---|
| I have read the Participant Information Sheet, have understood the nature of this research and why I have been selected. I have had the opportunity to ask questions and have them answered to my satisfaction | |
| I agree to take part in this study, and have been given sufficient time to consider whether or not to participate. | |
| I have had the opportunity to use support from whānau/family or a friend to help me ask questions and understand the study. | |
| I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time | |
| If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed *(please circle)* | Yes No |
| I understand that blood and stool samples will be collected and used for research. | |
| I wish for a karakia to be said at the time of my tissue disposal *(please circle)* | Yes No |
| I understand that any test results found to be outside the normal healthy range will be conveyed to me, and that if I do not wish to be informed I cannot participate in this study. | |
| I consent to my GP or current provider being informed about any significant abnormal results obtained during the study, with my permission *(please circle)* | Yes No |
| I agree to my stool samples being sent to an overseas laboratory (Beijing, China) and I am aware that these samples will be disposed of using established guidelines for discarding biohazard waste. | |
| I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study. | |

MEDICAL AND HEALTH SCIENCES

| | |
|---|---|
| I understand my responsibilities as a study participant, and know who to contact if I have any questions about the study in general. | |
| I wish to receive a summary of the results from the study *(please circle)* | Yes No |
| I understand that the results from this study will be used for scientific publication and presentations | |
| I agree not to restrict the use of any data or results that arise from this research, provided that such a use is only for scientific purposes. | |

**Declaration by participant:**
I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

**Declaration by member of research team:**

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

**APPROVED BY THE HEALTH AND DISABILITY ETHICS COMMITTEE ON 27/05/2022, Reference Number 2022 EXP 12513**

## Appendix 3: Dietary Screening Tool

| Dietary Screening Tool | |
|---|---|
| How often do you usually eat wholegrain breads or crackers? | ◯ Never OR less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you usually eat wholegrain cereals? (e.g. Weetbix, porridge, muesli) | ◯ Never OR less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat beef or lamb (including mince)? | ◯ Never or once a week or less<br>◯ 2-3 times a week<br>◯ 4-5 times a week<br>◯ More than 5 times per week |
| How often do you eat fish or seafood that IS NOT fried (including tinned)? | ◯ Never<br>◯ Less than once a week<br>◯ Once a week<br>◯ More than once per week |
| How many servings of LOW FAT milk, cheese, or yoghurt do you usually have each DAY? | ◯ None<br>◯ Once<br>◯ Two or more |
| How many different vegetable servings do you usually have at your main meal of the day? | ◯ None<br>◯ One<br>◯ Two or more |
| How often do you eat carrots, sweet potatoes, or pumpkin? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat rocket, spinach, or silverbeet? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat brocolli or cauliflower? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat fruit (not including juice)? Please include fresh, canned, or frozen fruit | ◯ Never or less than once a week<br>◯ 1 or 2 times a week<br>◯ 3-5 times a week<br>◯ Every day or almost every day |

| | |
|---|---|
| How often do you consume olive oil? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 to 5 times a week<br>◯ Every day or almost every day |
| How often do you consume legumes, such as lentils or chickpeas? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 to 5 times a week<br>◯ Every day or almost every day |
| How often do you consume fresh nuts, such as almonds, cashews, walnuts, or brazil nuts? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 to 5 times a week<br>◯ Every day or almost every day |
| How often do you drink (non-diet) soft drinks or cordials? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you usually eat sweets or chocolate? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you usually eat chips, twisties, or something similar? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat pies, sausage rolls, or hot chips? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat cakes, biscuits, ice creams, or doughnuts? | ◯ Never<br>◯ Less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat lunchmeats or deli meats (e.g. ham, bacon, or sausage)? | ◯ Never or less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |
| How often do you eat takeaway meals, such as McDonalds, KFC, Pizza Hut, or Burger King? | ◯ Never or less than once a week<br>◯ 1 or 2 times a week<br>◯ 3 or more times a week |

## Appendix 4a: State Trait Anxiety Inventory: State Subscale (STAI-S) Questionnaire

**LINK Trial** **STAI-S Form** **Test 1**

**DIRECTIONS**

- A number of statements which people have used to describe themselves are given below. Read each statement, then circle the appropriate number to the right of the statement to indicate how you **feel right now**, that is, **at this moment**.
- There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe your **present feelings** best.

| | Not at all | Somewhat | Moderately so | Very much so |
|---|---|---|---|---|
| 1. I feel calm | 1 | 2 | 3 | 4 |
| 2. I feel secure | 1 | 2 | 3 | 4 |
| 3. I am tense | 1 | 2 | 3 | 4 |
| 4. I feel strained | 1 | 2 | 3 | 4 |
| 5. I feel at ease | 1 | 2 | 3 | 4 |
| 6. I feel upset | 1 | 2 | 3 | 4 |
| 7. I am worrying over possible misfortunes | 1 | 2 | 3 | 4 |
| 8. I feel satisfied. | 1 | 2 | 3 | 4 |
| 9. I feel frightened | 1 | 2 | 3 | 4 |
| 10. I feel comfortable | 1 | 2 | 3 | 4 |
| 11. I feel self-confident | 1 | 2 | 3 | 4 |
| 12. I feel nervous | 1 | 2 | 3 | 4 |
| 13. I am jittery | 1 | 2 | 3 | 4 |
| 14. I feel indecisive | 1 | 2 | 3 | 4 |
| 15. I am relaxed | 1 | 2 | 3 | 4 |
| 16. I feel content | 1 | 2 | 3 | 4 |
| 17. I am worried | 1 | 2 | 3 | 4 |
| 18. I feel confused | 1 | 2 | 3 | 4 |
| 19. I feel steady | 1 | 2 | 3 | 4 |
| 20. I feel pleasant | 1 | 2 | 3 | 4 |

**Participant:** **Visit:** **Date/time:**

## Appendix 4b: Visual Analogue Mood Scale (VAMS) Questionnaire

**LINK Trial** **Visual Analogue Mood Scales** **Test 1**

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**DIRECTIONS**

1. Please rate the way you feel in terms of the dimensions given below
2. Regard the line as representing the full range of each dimension.
3. Rate your feelings as they are **AT THE MOMENT**
4. Mark clearly and perpendicularly (vertically) across each line.

---

| | | |
|---|---|---|
| Alert | ——— | Drowsy |
| Calm | ——— | Excited |
| Strong | ——— | Feeble |
| Muzzy | ——— | Clear-headed |
| Well-coordinated | ——— | Clumsy |
| Lethargic | ——— | Energetic |
| Contented | ——— | Discontented |
| Troubled | ——— | Tranquil |
| Mentally slow | ——— | Quick-witted |
| Tense | ——— | Relaxed |
| Attentive | ——— | Dreamy |
| Incompetent | ——— | Proficient |
| Happy | ——— | Sad |
| Antagonistic | ——— | Friendly |
| Interested | ——— | Bored |
| Withdrawn | ——— | Sociable |

**How stressed do you currently feel?**

| | | |
|---|---|---|
| Extremely | ——— | Not at all |

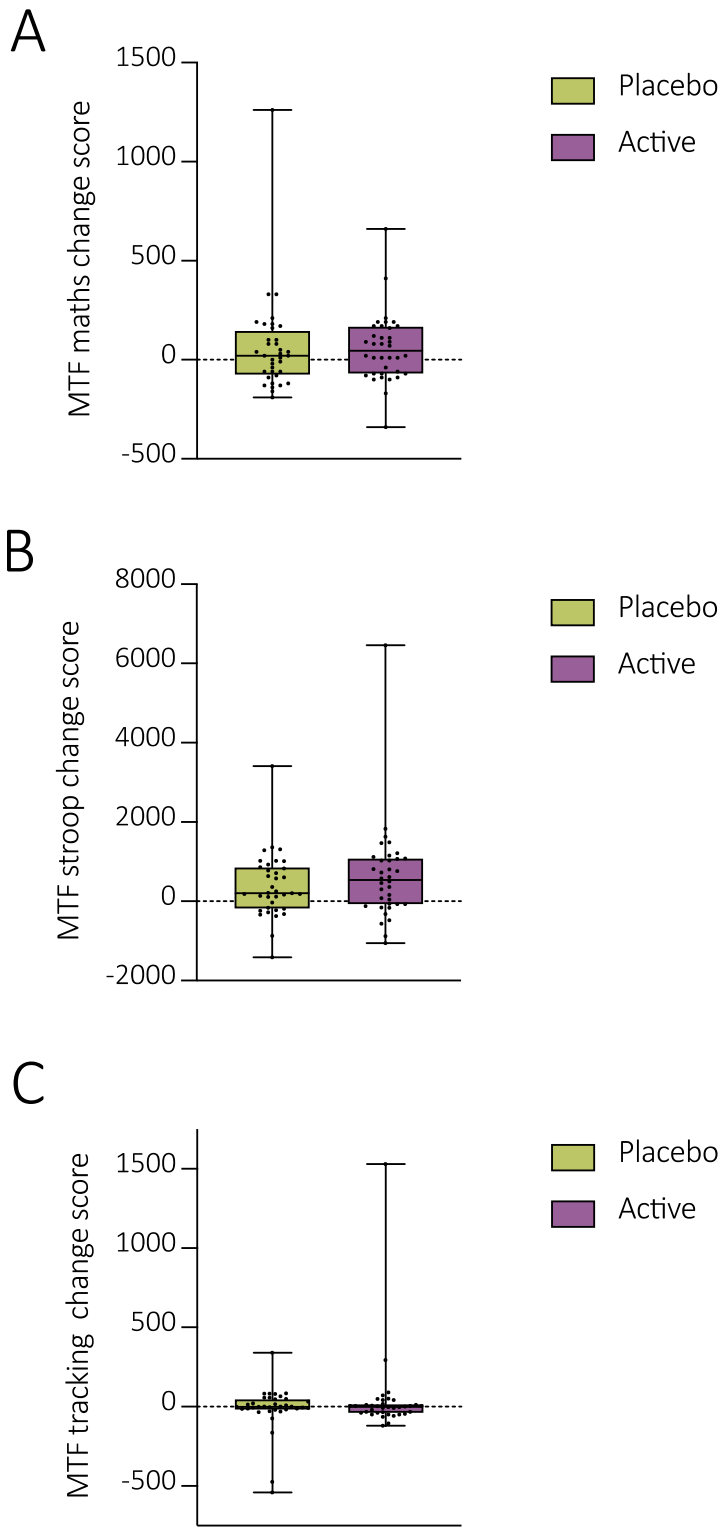
**How fatigued do you currently feel?**

| | | |
|---|---|---|
| Extremely | ——— | Not at all |

**Participant:** **Visit:** **Date/time:**

## Appendix 5: Effect of treatment on change in MTF maths, Stroop, and tracking scores

Data presented for A) Maths, B) Stroop, and C) Tracking




The MTF change score indicate the mean maths, Stroop, or tracking change scores between baseline and follow-up (4 weeks). P-values reflect a paired t-test comparing the placebo to active intervention change scores.

# References


1. World Health Organisation. Optimizing brain health across the life course [Internet]. Geneva: World Health Organisation; 2022. Available from: https://iris.who.int/bitstream/handle/10665/361251/9789240054561-eng.pdf?sequence=1
2. Tost H, Champagne FA, Meyer-Lindenberg A. Environmental influence in the brain, human welfare and mental health. Nat Neurosci. 2015 Oct;18(10):1421-31.
3. Ottersen OP. How hardwired is the brain? Technological advances provide new insight into brain malleability and neurotransmission. Nutr Rev. 2010;68(2):S60–4.
4. Scholey A. Nutrients for neurocognition in health and disease: Measures, methodologies and mechanisms. Proc Nutr Soc. 2018 Feb; 77(1):73–83.
5. Avan A, Hachinski V, Brain Health Learn and Act Group. Brain health: Key to health, productivity, and well-being. Alzheimers Dement. 2022 Jul 1;18(7):1396–407.
6. Hallam B, Petersen I, Cooper C, Avgerinou C, Walters K. Time trends in incidence of reported memory concerns and cognitive decline: A cohort study in UK primary care. Clin Epidemiol. 2022 Mar;14:395–408.
7. Wang Y, Pan Y, Li H. What is brain health and why is it important? BMJ. 2020 Oct 9;371.
8. Wimo A, Seeher K, Cataldi R, Cyhlarova E, Dielemann JL, Frisell O, et al. The worldwide costs of dementia in 2019. Alzheimers Dement. 2022;19(7):2865-73.
9. Millan MJ, Agid Y, Brüne M, Bullmore ET, Carter CS, Clayton NS, et al. Cognitive dysfunction in psychiatric disorders: Characteristics, causes and the quest for improved therapy. Nat Rev Drug Discov. 2012;11(2):141–68.
10. Handel MJ. Trends in job skill demands in OECD countries. 2012;(143). Available from: http://dx.doi.org/10.1787/5k8zk8pcq6td-en
11. Turner AI, Smyth N, Hall SJ, Torres SJ, Hussein M, Jayasinghe SU, et al. Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence. Psychoneuroendocrinology. 2020 Apr;114.
12. World Health Organization. World mental health report: transforming mental health for all. Geneva: World Health Organisation; 2022.
13. Hanrahan NP, Stuart GW, Delaney KR, Wilson C. Mental health is an urgent public health concern. Nurs Outlook. 2013 May;61(3):185–6.
14. Ngui EM, Khasakhala L, Ndetei D, Roberts LW. Mental disorders, health inequalities and ethics: A global perspective. Int Rev of Psychiatry. 2010;22(3):235–44.
15. Gomez-Pinilla F, Nguyen TTJ. Natural mood foods: The actions of polyphenols against psychiatric and cognitive disorders. Nutr Neurosci. 2012 May;15(3):127–33.
16. Kent K, Charlton KE, Netzel M, Fanning K. Food-based anthocyanin intake and cognitive outcomes in human intervention trials: a systematic review. J Hum Nutr Diet. 2017 Jun;30(3):260–74.
17. Jacka FN, Pasco JA, Mykletun A, Williams LJ, Hodge AM, O'Reilly SL, et al. Association of Western and traditional diets with depression and anxiety in women. 2010 Mar;167(3): 305-11.
18. Sarris J, Logan AC, Akbaraly TN, Amminger GP, Balanzá-Martínez V, Freeman MP, et al. Nutritional medicine as mainstream in psychiatry. Lancet Psychiatry. 2015;2(3): 271–4.

19. O'Neil A, Quirk SE, Housden S, Brennan SL, Williams LJ, Pasco JA, et al. Relationship between diet and mental health in children and adolescents: A systematic review. Am J Public Health. 2014;104(10).
20. Lai JS, Hiles S, Bisquera A, Hure AJ, McEvoy M, Attia J. A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. Am J Clin Nutr. 2014 Jan 1;99(1):181–97.
21. Jacka FN, O'Neil A, Opie R, Itsiopoulos C, Cotton S, Mohebbi M, et al. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). BMC Med. 2017 Jan 30;15(1).
22. Petersson SD, Philippou E. Mediterranean diet, cognitive function, and dementia: A systematic review of the evidence. Vol. Adv Nutr. 2016;7(5)889–904.
23. Francis H, Stevenson R. The longer-term impacts of Western diet on human cognition and the brain. Appetite. 2013 Apr;63:119–28.
24. McEvoy CT, Leng Y, Peeters GM, Kaup AR, Allen IE, Yaffe K. Interventions involving a major dietary component improve cognitive function in cognitively healthy adults: a systematic review and meta-analysis. Nutr Res. 2019;66:1–12.
25. Esgunoglu L, Jennings A, Connole ES, Murphy KJ, Minihane AM. Short-Term effects of a Mediterranean-style dietary pattern on cognition and mental well-being: A systematic review of clinical trials. Br J Nutr. 2022;128(7):1247-1256.
26. Scholey AB, French SJ, Morris PJ, Kennedy DO, Milne AL, Haskell CF. Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. J Psychopharmacol. 2010 Oct;24(10):1505–14.
27. Yang W, Cui K, Li X, Zhao J, Zeng Z, Song R, et al. Effect of polyphenols on cognitive function: Evidence from population-based studies and clinical trials. J Nutr Health Aging. 2021;25(10):1190–204.
28. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. 2009;2(5):270-278.
29. Cásedas G, González-Burgos E, Smith C, López V, Gómez-Serranillos MP. Regulation of redox status in neuronal SH-SY5Y cells by blueberry (Vaccinium myrtillus L.) juice, cranberry (Vaccinium macrocarpon A.) juice and cyanidin. Food Chem Toxicol. 2018 Aug;118:572–80.
30. Silva RFM, Pogačnik L. Polyphenols from food and natural products: Neuroprotection and safety. Antioxidants. 2020 Jan;9(1):61.
31. Rasouli H, Farzaei MH, Khodarahmi R. Polyphenols and their benefits: A review. Int J Food Prop. 2017;20:1700–41.
32. Martín MÁ, Ramos S. Dietary flavonoids and insulin signaling in diabetes and obesity. Cells. 2021 Jun;10(6):1474.
33. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004 May;79(5):727-47.
34. Williamson G. The role of polyphenols in modern nutrition. Nutr Bull. 2017;42(3):226–35.
35. Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, et al. Natural polyphenols: An overview. Inter J Food Prop. 2017;20(8):1689–99.
36. Spagnuolo C, Napolitano M, Tedesco I, Moccia S, Milito A, Russo GL. Neuroprotective role of natural polyphenols. Curr Top Med Chem. 2016;16(17):1943-50.
37. Tsuda T. Dietary anthocyanin-rich plants- biochemical basis and recent progress in health benefits studies. Mol Nutr Food Res. 2012 Jan;56(1):159-70.

38. Medina dos Santos N, Berilli Batista P, Batista ÂG, Maróstica Júnior MR. Current evidence on cognitive improvement and neuroprotection promoted by anthocyanins. Curr Opin Food Sci. 2019;26:71–8.
39. Ma H, Johnson SL, Liu W, Dasilva NA, Meschwitz S, Dain JA, et al. Evaluation of polyphenol anthocyanin-enriched extracts of blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry for free radical scavenging, reactive carbonyl species trapping, anti-glycation, anti-β-amyloid aggregation, and microglial neuroprotective effects. Int J Mol Sci. 2018 Feb 3;19(2).
40. Pogačnik L, Pirc K, Palmela I, Skrt M, Kwang KS, Brites D, et al. Potential for brain accessibility and analysis of stability of selected flavonoids in relation to neuroprotection in vitro. Brain Res. 2016 Nov 15;1651:17–26.
41. Winter AN, Brenner MC, Punessen N, Snodgrass M, Byars C, Arora Y, et al. Comparison of the neuroprotective and anti-Inflammatory effects of the anthocyanin metabolites, protocatechuic acid and 4-hydroxybenzoic acid. Oxid Med Cell Longev. 2017 Jun;2017.
42. Amin FU, Shah SA, Badshah H, Khan M, Kim MO. Anthocyanins encapsulated by PLGA@PEG nanoparticles potentially improved its free radical scavenging capabilities via p38/JNK pathway against Aβ1-42-induced oxidative stress. J Nanobiotechnology. 2017 Feb 7;15(1):12.
43. Andrade PB, Grosso C, Valentão P, Bernardo J. Flavonoids in neurodegeneration: Limitations and strategies to cross CNS barriers. Curr Med Chem. 2016;23(36):4151-74.
44. Palmer AM. The blood-brain barrier. Neurobiol Dis. 2010 Jan;37(1):1–2.
45. Faria A, Meireles M, Fernandes I, Santos-Buelga C, Gonzalez-Manzano S, Dueñas M, et al. Flavonoid metabolites transport across a human BBB model. Food Chem. 2014 Apr;149:190–6.
46. Spencer JPE. Beyond antioxidants: The cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. Proc Nutr Soc. 2010 May;69(2):244–60.
47. Kim MJ, Rehman SU, Amin FU, Kim MO. Enhanced neuroprotection of anthocyanin-loaded PEG-gold nanoparticles against Aβ1-42-induced neuroinflammation and neurodegeneration via the NF-KB /JNK/GSK3β signaling pathway. Nanomedicine. 2017 Nov 1;13(8):2533–44.
48. Thummayot S, Tocharus C, Suksamrarn A, Tocharus J. Neuroprotective effects of cyanidin against Aβ-induced oxidative and ER stress in SK-N-SH cells. Neurochem Int. 2016 Dec 1;101:15–21.
49. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, et al. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol. 2012 May;71(5):362-81.
50. González-Reyes RE, Nava-Mesa MO, Vargas-Sánchez K, Ariza-Salamanca D, Mora-Muñoz L. Involvement of astrocytes in Alzheimer's disease from a neuroinflammatory and oxidative stress perspective. Front Mol Neurosci. 2017 Dec;10:427.
51. Hornedo-Ortega R, Álvarez-Fernández MA, Cerezo AB, Richard T, Troncoso AM, Garcia-Parrilla MC. Protocatechuic acid: Inhibition of fibril formation, destabilization of preformed fibrils of amyloid-β and α-Synuclein, and neuroprotection. J Agric Food Chem. 2016 Oct;64(41):7722–32.
52. Vicente Miranda H, El-Agnaf OM, Outeiro TF. Glycation in Parkinson's disease and Alzheimer's disease. Mov Disord. 2016 Jun;31(6):782-90.

53. Angeloni C, Zambonin L, Hrelia S. Role of methylglyoxal in Alzheimer's disease. BioMed Res Int. 2014 Mar.
54. Schnaider Beeri M, Silverman J, Moshier E, Schmeidler J, Godbold J, Uribarri J, et al. Serum concentration of an inflammatory glycotoxin, methylglyoxal, is associated with increased cognitive decline in elderly individuals. Mech Ageing Dev. 2011;132(11-12):583-7.
55. De Ferrars RM, Czank C, Zhang Q, Botting NP, Kroon PA, Cassidy A, et al. The pharmacokinetics of anthocyanins and their metabolites in humans. Br J Pharmacol. 2014;171(13):3268–82.
56. Carvalho FB, Gutierres JM, Bohnert C, Zago AM, Abdalla FH, Vieira JM, et al. Anthocyanins suppress the secretion of proinflammatory mediators and oxidative stress, and restore ion pump activities in demyelination. J Nutr Biochem. 2015 Apr 1;26(4):378–90.
57. Bowtell JL, Aboo-Bakkar Z, Conway ME, Adlam ALR, Fulford J. Enhanced task-related brain activation and resting perfusion in healthy older adults after chronic blueberry supplementation. App Physiol Nutr Metab. 2017;42(7):773–9.
58. Bookheimer SY, Renner BA, Ekstrom A, Li Z, Henning SM, Brown JA, et al. Pomegranate juice augments memory and fMRI activity in middle-aged and older adults with mild memory complaints. Evid Based Complement Alternat Med. 2013;2013.
59. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD, et al. Concord grape juice supplementation and neurocognitive function in human aging. J Agric Food Chem. 2012 Jun 13;60(23):5736–42.
60. Spencer JPE. The impact of flavonoids on memory: Physiological and molecular considerations. Chem Soc Rev. 2009 Mar;38(4):1152–61.
61. Spencer JPE. The impact of fruit flavonoids on memory and cognition. Br J Nutr. 2010 Oct;104:S40-S47.
62. Juan Z, Lu J, Wang X, Wang X, Hu W, Hong F, et al. Purple sweet potato color protects against high-fat diet-induced cognitive deficits through AMPK-mediated autophagy in mouse hippocampus. J Nutr Biochem. 2019 Mar;65:35–45.
63. Carey AN, Gildawie KR, Rovnak A, Thangthaeng N, Fisher DR, Shukitt-Hale B. Blueberry supplementation attenuates microglia activation and increases neuroplasticity in mice consuming a high-fat diet. Nutr Neurosci. 2019 Apr 3;22(4):253–63.
64. Jia Y, Dong Z, Yan T, Wu B, Liao Z, Bi K, et al. Antidepressant-like activity of red wine phenolic extracts in repeated corticosterone-induced depression mice via BDNF/TrkB/CREB signaling pathway. BIO Web Conf. 2016;7.
65. Rendeiro C, Vauzour D, Rattray M, Waffo-Téguo P, Mérillon JM, Butler LT, et al. Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. PLoS One. 2013 May 28;8(5).
66. Goldsmith HS. Alzheimer's disease: A decreased cerebral blood flow to critical intraneuronal elements is the cause. J Alzheimers Dis. 2022;85(4):1419–22.
67. Lamport DJ, Williams CM. Polyphenols and cognition in humans: An overview of current evidence from recent systematic reviews and meta-analyses. Brain Plast. 2021;6(2):139–53.
68. Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, et al. Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci. 1998;18(19):8047-55.

69. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, Mcewen JJ, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci. 1999;19(18):8114-21.
70. Talavéra S, Felgines C, Texier O, Besson C, Gil-Izquierdo A, Lamaison JL, et al. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. J Agric Food Chem. 2005 May;53(10):3902–8.
71. Youdim KA, Shukitt-Hale B, Joseph JA. Flavonoids and the brain: Interactions at the blood-brain barrier and their physiological effects on the central nervous system. Free Radic Biol Med. 2004;37(11):1683–93.
72. Wei J, Zhang G, Zhang X, Xu D, Gao J, Fan J, et al. Anthocyanins from Black Chokeberry (Aroniamelanocarpa Elliot) delayed aging-related degenerative changes of brain. J Agric Food Chem. 2017 Jul;65(29):5973–84.
73. Ali T, Kim T, Rehman SU, Khan MS, Amin FU, Khan M, et al. Natural dietary supplementation of anthocyanins via PI3K/Akt/Nrf2/HO-1 pathways mitigate oxidative stress, neurodegeneration, and memory impairment in a mouse model of Alzheimer's disease. Mol Neurobiol. 2018 Jul;55(7):6076–93.
74. Shukitt-Hale B, Cheng V, Joseph JA. Effects of blackberries on motor and cognitive function in aged rats. Nutr Neurosci. 2009 Jun;12(3):135–40.
75. Thangthaeng N, Poulose SM, Gomes SM, Miller MG, Bielinski DF, Shukitt-Hale B. Tart cherry supplementation improves working memory, hippocampal inflammation, and autophagy in aged rats. Age (Dordr). 2016 Dec;38(5–6):393–404.
76. Williams CM, El Mohsen MA, Vauzour D, Rendeiro C, Butler LT, Ellis JA, et al. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. Free Radic Biol Med. 2008 Aug 1;45(3):295–305.
77. Goyarzu P, Malin DH, Lau FC, Taglialatela G, Moon WD, Jennings R, et al. Blueberry supplemented diet: Effects on object recognition memory and nuclear factor-kappa B levels in aged rats. Nutr Neurosci. 2004 Apr;7(2):75–83.
78. Barros D, Amaral OB, Izquierdo I, Geracitano L, do Carmo Bassols Raseira M, Henriques AT, et al. Behavioral and genoprotective effects of Vaccinium berries intake in mice. Pharmacol Biochem Behav. 2006 Jun;84(2):229–34.
79. Shukitt-Hale B, Smith DE, Meydani M, Joseph JA. The effects of dietary antioxidants on psychomotor performance in aged mice. Exp Gerontol. 1999 Sep;34(6):797-808.
80. Shukitt-Hale B, Carey A, Simon L, Mark DA, Joseph JA. Effects of Concord grape juice on cognitive and motor deficits in aging. Nutrition. 2006 Mar;22(3):295–302.
81. Van Praag H, Lucero MJ, Yeo GW, Stecker K, Heivand N, Zhao C, et al. Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. J Neurosci. 2007 May 30;27(22):5869–78.
82. Khan MS, Ali T, Kim MW, Jo MH, Jo MG, Badshah H, et al. Anthocyanins protect against LPS-induced oxidative stress-mediated neuroinflammation and neurodegeneration in the adult mouse cortex. Neurochem Int. 2016 Nov 1;100:1–10.
83. Khan MS, Ali T, Kim MW, Jo MH, Chung J Il, Kim MO. Anthocyanins improve hippocampus-dependent memory function and prevent neurodegeneration via JNK/Akt/GSK3β signaling in LPS-treated adult mice. Mol Neurobiol. 2019 Jan 1;56(1):671–87.

84. Rehman SU, Shah SA, Ali T, Chung J Il, Kim MO. Anthocyanins reversed D-galactose-induced oxidative stress and neuroinflammation mediated cognitive impairment in adult rats. Mol Neurobiol. 2017 Jan 1;54(1):255–71.
85. Nurk E, Refsum H, Drevon CA, Tell GS, Nygaard HA, Engedal K, et al. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. J Nutrition. 2009;139(1):120–7.
86. Yeh TS, Yuan C, Ascherio A, Rosner BA, Willett WC, Blacker D. Long-term dietary flavonoid intake and subjective cognitive decline in US men and women. Neurology. 2021 Sep 7;97(10):E1041–56.
87. Shishtar E, Rogers GT, Blumberg JB, Au R, Jacques PF. Long-term dietary flavonoid intake and risk of Alzheimer disease and related dementias in the Framingham Offspring Cohort. Am J Clin Nutr. 2020 Aug 1;112(2):343–53.
88. Kent K, Charlton K, Roodenrys S, Batterham M, Potter J, Traynor V, et al. Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. Eur J Nutr. 2017 Feb 1;56(1):333–41.
89. Igwe EO, Charlton KE, Roodenrys S, Kent K, Fanning K, Netzel ME. Anthocyanin-rich plum juice reduces ambulatory blood pressure but not acute cognitive function in younger and older adults: a pilot crossover dose-timing study. Nutr Res. 2017 Nov 1;47:28–43.
90. Calapai G, Bonina F, Bonina A, Rizza L, Mannucci C, Arcoraci V, et al. A randomized, double-blinded, clinical trial on effects of a Vitis vinifera extract on cognitive function in healthy older adults. Front Pharmacol. 2017 Oct 31;8:776.
91. Boespflug EL, Eliassen JC, Dudley JA, Shidler MD, Kalt W, Summer SS, et al. Enhanced neural activation with blueberry supplementation in mild cognitive impairment. Nutr Neurosci. 2018 Apr 21;21(4):297–305.
92. Lamport DJ, Lawton CL, Merat N, Jamson H, Myrissa K, Hofman D, et al. Concord grape juice, cognitive function, and driving performance: A 12-wk, placebo-controlled, randomized crossover trial in mothers of preteen children. Am J Clin Nutr. 2016 Mar 1;103(3):775–83.
93. Whyte AR, Schafer G, Williams CM. Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. Eur J Nutr. 2016 Sep 1;55(6):2151–62.
94. Whyte AR, Schafer G, Williams CM. The effect of cognitive demand on performance of an executive function task following wild blueberry supplementation in 7 to 10 years old children. Food Funct. 2017 Nov 1;8(11):4129–38.
95. Whyte AR, Williams CM. Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10y old children. Nutrition. 2015 Mar 1;31(3):531–4.
96. Dodd GF, Williams CM, Butler LT, Spencer JPE. Acute effects of flavonoid-rich blueberry on cognitive and vascular function in healthy older adults. Nutr Healthy Aging. 2019;5(2):119–32.
97. Khalid S, Barfoot KL, May G, Lamport DJ, Reynolds SA, Williams CM. Effects of acute blueberry flavonoids on mood in children and young adults. Nutrients. 2017 Feb 20;9(2).
98. Barfoot KL, May G, Lamport DJ, Ricketts J, Riddell PM, Williams CM. The effects of acute wild blueberry supplementation on the cognition of 7–10-year-old schoolchildren. Eur J Nutr. 2019 Oct 1;58(7):2911–20.
99. Caldwell K, Charlton KE, Roodenrys S, Jenner A. Anthocyanin-rich cherry juice does not improve acute cognitive performance on RAVLT. Nutr Neurosci. 2016 Nov;19(9): 423–4.

100. Watson AW, Haskell-Ramsay CF, Kennedy DO, Cooney JM, Trower T, Scheepens A. Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults. J Funct Foods. 2015 Aug 1;17:524–39.
101. Philip P, Sagaspe P, Taillard J, Mandon C, Constans J, Pourtau L, et al. Acute intake of a grape and blueberry polyphenol-rich extract ameliorates cognitive performance in healthy young adults during a sustained cognitive effort. Antioxidants (Basel). 2019;8(12):650.
102. Watson AW, Okello EJ, Brooker HJ, Lester S, McDougall GJ, Wesnes KA. The impact of blackcurrant juice on attention, mood and brain wave spectral activity in young healthy volunteers. Nutr Neurosci. 2019 Aug 3;22(8):596–606.
103. Whyte AR, Cheng N, Fromentin E, Williams CM. A randomized, double-blinded, placebo-controlled study to compare the safety and efficacy of low dose enhanced wild blueberry powder and wild blueberry extract (Thinkblue™) in maintenance of episodic and working memory in older adults. Nutrients. 2018 Jun 1;10(6).
104. Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, et al. Blueberry supplementation improves memory in older adults. J Agric Food Chem. 2010 Apr 14;58(7):3996–4000.
105. Miller MG, Hamilton DA, Joseph JA, Shukitt-Hale B. Dietary blueberry improves cognition among older adults in a randomized, double-blind, placebo-controlled trial. Eur J Nutr. 2018 Apr 1;57(3):1169–80.
106. Travica N, D'Cunha NM, Naumovski N, Kent K, Mellor DD, Firth J, et al. The effect of blueberry interventions on cognitive performance and mood: A systematic review of randomized controlled trials. Brain Behav Immun. 2020; 85:96–105.
107. Gibson N, Baker D, Sharples A, Braakhuis A. Improving mental performance in an athletic population with the use of Ārepa®, a blackcurrant based nootropic drink: A randomized control trial. Antioxidants. 2020 Apr 1;9(4).
108. Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B, Joseph JA. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. Br J Nutr. 2010 Mar;103(5):730–4.
109. Pipingas A, Silberstein RB, Vitetta L, Rooy C Van, Harris E V, Young JM, et al. Improved cognitive performance after dietary supplementation with a Pinus radiata bark extract formulation. Phytother Res. 2008;22:1168–74.
110. Cook MD, Sandu, BSc AK, Joyce, PhD JP. Effect of New Zealand blackcurrant on blood pressure, cognitive function and functional performance in older adults. J Nutr Gerontol Geriatr. 2020 Apr 2;39(2):99–113.
111. Ahles S, Stevens YR, Joris PJ, Vauzour D, Adam J, Groot E de, et al. The effect of long-term Aronia melanocarpa extract supplementation on cognitive performance, mood, and vascular function: A randomized controlled trial in healthy, middle-aged individuals. Nutrients. 2020 Aug 1;12(8):1–16.
112. Kimble R, Keane KM, Lodge JK, Cheung W, Haskell-Ramsay CF, Howatson G. Polyphenol-rich tart cherries (Prunus Cerasus, cv Montmorency) improve sustained attention, feelings of alertness and mental fatigue and influence the plasma metabolome in middle-aged adults: a randomised, placebo-controlled trial. Br J Nutr. 2022 Dec 28;128(12):2409–20.

113. Young LM, Gauci S, Scholey A, White DJ, Pipingas A. self-selection bias: An essential design consideration for nutrition trials in healthy populations. Front Nutr. 2020 Nov 10;7.
114. Bayes J, Schloss J, Sibbritt D. Effects of polyphenols in a Mediterranean diet on symptoms of depression: A systematic literature review. Adv Nutr. 2020;11(3):602–15.
115. Lin K, Li Y, Toit E Du, Wendt L, Sun J. Effects of polyphenol supplementations on improving depression, anxiety, and quality of life in patients with depression. Front Psychiatry. 2021;12.
116. Fisk J, Khalid S, Reynolds SA, Williams CM. Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents. Br J Nutr. 2020;124(2):181–8.
117. Blackcurrants New Zealand. The NZ Blackcurrant Industry [Internet]. 2023 [cited 2023 Dec 12]. Available from: https://www.blackcurrant.co.nz/blackcurrants/the-nz-industry/
118. Braakhuis AJ, Somerville VX, Hurst RD. The effect of New Zealand blackcurrant on sport performance and related biomarkers: A systematic review and meta-analysis. J Int Soc Sports Nutr. 2020;17(1):25.
119. Ali Redha A, Anusha Siddiqui S, Zare R, Spadaccini D, Guazzotti S, Feng X, et al. Blackcurrants: A nutrient-rich source for the development of functional foods for improved athletic performance. Food Rev Int. 2022;40(1).
120. Marra MV, Thuppal S V., Johnson EJ, Bailey RL. Validation of a dietary screening tool in a middle-aged appalachian population. Nutrients. 2018 Mar 12;10(3).
121. Bailey RL, Miller PE, Mitchell DC, Hartman TJ, Lawrence FR, Sempos CT, et al. Dietary screening tool identifies nutritional risk in older adults. Am J Clin Nutr. 2009 Jul 1;90(1):177–83.
122. Advanced Micro Devices (AMD). True Random Number Service [Internet]. AMD;2023. Available from: https://docs.amd.com/r/en-US/ug1304-versal-acap-ssdg/True-Random-Number-Generator
123. Wetherell MA, Carter K. The multitasking framework: The effects of increasing workload on acute psychobiological stress reactivity. Stress Health. 2014;30(2):103–9.
124. Wetherell MA, Craw O, Smith K, Smith MA. Psychobiological responses to critically evaluated multitasking. Neurobiol Stress. 2017 Dec 1;7:68–73.
125. Scholey A, Haskell C, Robertson B, Kennedy D, Milne A, Wetherell M. Chewing gum alleviates negative mood and reduces cortisol during acute laboratory psychological stress. Physiol Behav. 2009 Jun 22;97(3–4):304–12.
126. Scholey A, Savage K, O'Neill B V., Owen L, Stough C, Priestley C, et al. Effects of two doses of glucose and a caffeine-glucose combination on cognitive performance and mood during multi-tasking. Hum Psychopharmacol. 2014 Sep 1;29(5):434–45.
127. White DJ, de Klerk S, Woods W, Gondalia S, Noonan C, Scholey AB. Anti-stress, behavioural and magnetoencephalography effects of an l-theanine-based nutrient drink: A randomised, double-blind, placebo-controlled, crossover trial. Nutrients. 2016 Jan 19;8(1).
128. Rossi V, Pourtois G. Transient state-dependent fluctuations in anxiety measured using STAI, POMS, PANAS or VAS: A comparative review. Anxiety Stress Coping. 2012 Nov;25(6):603–45.

129. Hidese S, Ogawa S, Ota M, Ishida I, Yasukawa Z, Ozeki M, et al. Effects of L-Theanine administration on stress- related symptoms and cognitive functions in healthy adults: A randomized controlled trial. Nutrients. 2019 Oct 1;11(10).
130. Ministry of Health New Zealand. Eating and activity guidelines for New Zealand adults. Wellington: Ministry of Health New Zealand; 2020.
131. Ministry of Health. Ethnicity data protocols for the health and disability sector. Ministry of Health; 2004.
132. National Health and Medical Research Council, Australian Government Department of Health and Ageing, New Zealand Ministry of Health. Nutrient reference values for Australia and New Zealand. Canberra: National Health and Medical Research Council; 2006.
133. World Health Organisation. WHO global report on sodium intake reduction [Internet]. 2023. Available from: https://www.who.int/publications/book-orders.
134. Young LM, Gauci S, Scholey A, White DJ, Lassemillante AC, Meyer D, et al. Self-reported diet quality differentiates nutrient intake, blood nutrient status, mood, and cognition: Implications for identifying nutritional neurocognitive risk factors in middle age. Nutrients. 2020 Oct 1;12(10):1–20.
135. Young LM, Gauci S, Arnoldy L, Martin L, Perry N, White DJ, et al. Investigating the effects of a multinutrient supplement on cognition, mood and biochemical markers in middle-aged adults with 'optimal' and 'sub-optimal' diets: A randomized double blind placebo controlled trial. Nutrients. 2022 Dec 1;14(23).
136. Baba Y, Inagaki S, Nakagawa S, Kaneko T, Kobayashi M, Takihara T. Effects of l-theanine on cognitive function in middle-aged and older subjects: a randomized placebo-controlled study. J Med Food. 2021 Apr 1;24(4):333–41.
137. Unno K, Tanida N, Ishii N, Yamamoto H, Iguchi K, Hoshino M, et al. Anti-stress effect of theanine on students during pharmacy practice: Positive correlation among salivary α-amylase activity, trait anxiety and subjective stress. Pharmacol Biochem Behav. 2013;111:128–35.
138. Gomez-Ramirez M, Kelly SP, Montesi JL, Foxe JJ. The effects of l-theanine on alpha-band oscillatory brain activity during a visuo-spatial attention task. Brain Topogr. 2009 Jun;22(1):44–51.
139. Kahathuduwa CN, Dassanayake TL, Amarakoon AMT, Weerasinghe VS. Acute effects of theanine, caffeine and theanine–caffeine combination on attention. Nutr Neurosci. 2017 Jul 3;20(6):369–77.
140. Joris PJ, Mensink RP, Adam TC, Liu TT. Cerebral blood flow measurements in adults: A review on the effects of dietary factors and exercise. Nutrients. 2018;10(5):530.
141. Ayaz M, Sadiq A, Junaid M, Ullah F, Ovais M, Ullah I, et al. Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. Front Aging Neurosci. 2019 Jun;11:155.
142. Kimura K, Ozeki M, Juneja LR, Ohira H. l-Theanine reduces psychological and physiological stress responses. Biol Psychol. 2007 Jan;74(1):39–45.
143. Lu K, Gray MA, Oliver C, Liley DT, Harrison BJ, Bartholomeusz CF, et al. The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans. Hum Psychopharmacol. 2004 Oct;19(7):457–65.
144. Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. Nutr Rev. 2014 Jan;72(1):48–54.

145. Theadom A, Mahon S, Barker-Collo S, Mcpherson K, Rush E, Vandal AC, et al. Enzogenol for cognitive functioning in traumatic brain injury: A pilot placebo-controlled RCT. Eur J Neurol. 2013 Aug;20(8):1135–44.
146. Williams RJ, Spencer JPE. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med. 2012;52(1):35–45.
147. Baroni L, Sarni AR, Zuliani C. Plant foods rich in antioxidants and human cognition: A systematic review. Antioxidants (Basel). 2021 Apr;10(5):714.
148. Grabska-Kobyłecka I, Szpakowski P, Król A, Książek-Winiarek D, Kobyłecki A, Głąbiński A, et al. Polyphenols and their impact on the prevention of neurodegenerative diseases and development. Nutrients. 2023;15(15):3454.
149. Ali T, Kim MJ, Rehman SU, Ahmad A, Kim MO. Anthocyanin-loaded PEG-gold nanoparticles enhanced the neuroprotection of anthocyanins in an Aβ1–42 mouse model of Alzheimer's disease. Mol Neurobiol. 2017 Oct 1;54(8):6490–506.