The Effects of Wild Blueberry Flavonoids on Mood and Cognition in Young Adults.

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

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Declaration

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

The work in this thesis is made up of 4 papers authored by me, my supervisors, Professor Shirley Reynolds and Professor Claire Williams, Katie Barfoot (co-author on study 3) and Jennifer Fisk (co-author on study 5). To clarify my specific contributions to the papers, I confirm that data used in all the papers was primarily obtained by me. I independently recruited young adults, handled data, conducted analysis, literature searches and completed the write up of all manuscript in this thesis. Children data in study 2 was collected, analysed and written by Katie Barfoot. Jennifer Fisk contributed equally in the data collection and analysis and write up for manuscript of the Chronic study (study 5) under consideration for publication. Authors/Supervisors Professor Shirley Reynolds and Professor Claire Williams assisted with the preparation of manuscripts.

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Abstract

Following the recent interest in the relationship between nutrition and depression, in exploring prevention and treatment strategies for mental health through dietary interventions, this thesis investigates the acute and chronic benefits of wild blueberry flavonoid supplementation on depressive symptoms and cognition in young adults (12-25 year olds).

Study 1, a systematic review, revealed that diets containing higher amount of fruits and vegetables have been shown to be inversely associated with symptoms of depression in children, young and older adults. Several micronutrients such as vitamins and minerals present in fruits and vegetables have been investigated regarding their effects on depressive symptoms. However, research on the effects of consumption of flavonoids, that have significant positive effects on cognitive functioning and are abundantly present in fruits and vegetables, on depressive symptoms has been limited and is virtually non-existent in young adults. Given the strong evidence showing the association between cognition and depression, we hypothesised that the beneficial effects of fruit and vegetable rich diets to alleviate symptoms of depression may be via flavonoid related improvement in cognition. However, the direct effects of flavonoids on mood has been unexplored. The collective studies in this thesis attempts to fill this gap in the literature.

Study 2 revealed that in 11-17 year olds (N = 77) there was no association between diet quality and depressive symptoms, however the amount of fruits and vegetables consumed was significantly lower than the recommended amounts. Further, more than half the adolescents consumed empty calories above the recommended amount, and none achieved the suggested ratio of carbohydrate to protein to fat in their diet. This in addition to the findings from the systematic review provided a rationale to explore the effects of flavonoids on symptoms of depression and cognitive ability. The acute (2 hours) psychological and cognitive effects of flavonoids were investigated across 2 double-blinded placebo-controlled crossover experiments (Study 3 and 4) in young adults (18-25-year-olds). Study 3 was a crossover study in 21 young adults. No intervention related improvement in cognition or negative affect was observed however, there was a significant improvement in positive affect after the consumption of wild blueberry drinks in Study 3. However, in this study participants' baseline mood was not accounted for, therefore, the study was repeated (Study 4) in 33 young adults who were screened for depressive symptoms at baseline. No significant influence of flavonoid rich blueberry drink was observed on

positive and negative affect or on any of the cognitive measures in healthy young adults or those with elevated symptoms of depression. This comparison requires replication due to the sample size of those with depressive symptoms being small (n=12).

Study 5, last in the series of studies is a randomised double-blinded placebo-controlled trial that investigated the effects of 4-week daily wild blueberry (253mg anthocyanins) intervention on transient and chronic mood and cognition in young adults (12-18 year olds). No significant beneficial effect of wild blueberry intervention was observed on transient mood and cognition after 2 and 4 weeks of the intervention. However, participants reported significantly fewer depression symptoms on the Mood and Feeling Questionnaire, after 4 weeks of daily wild blueberry supplementation.

The work in this thesis demonstrates that there is low consumption of fruits and vegetables (therefore, low flavonoid intake) in young adults and that, the administration of a flavonoid rich wild blueberry supplementation, can result in an elevated positive affect within 2 hours and decreased depressive symptoms after 4 weeks of daily supplementation. Overall, these studies are the first to show that dietary flavonoids have the potential to affect transient and chronic mood in young adults. However, these studies require replication, especially in young people with elevated symptoms of depression and in participants with a diagnosis of depression. If replicated, blueberry flavonoid supplementation could be a potential prevention or early intervention strategy for young adults at risk of and suffering from depression and have benefits for public mental health.

Thesis Outline

The aim of this doctoral thesis is to investigate the effects of dietary nutrients, particularly flavonoid-rich blueberries, on mood in adolescents and young adults. Affective disorders, including depression, are common mental health problems and major depressive disorder is one of the leading causes of disability globally (World Health Organisation, 2018). Treatments for depression are difficult to access in most parts of the world. Therefore, exploring preventative strategies and new evidence-based treatments is vital. This thesis addresses a key gap in the literature about the effects of diet, especially dietary flavonoids, on mental health. The following section briefly describes the aims and objectives of each chapter to give an overview of the thesis.

Chapter 1 is a general introduction to major depressive disorder discussing its causes and impacts on adolescents and young adults. Depressive disorders in adolescents are an important public health concern as they are extremely prevalent, affect quality of life and are a risk factor for suicide. In addition, depression has many lifelong negative impacts, including loss of cognitive, social, and interpersonal skills, under-employment, and poor physical health. Adolescents with depression are likely to relapse during adulthood and are at greater risk of developing personality disorders and substance abuse in adult life. In this chapter, current evidence-based treatments for adolescent depression will be described and potential barriers to effective care highlighted.

Chapter 2 critically evaluates the evidence of an association between individual macro and micronutrients, including carbohydrates, proteins, and vitamins, on depression. The proposed mechanisms behind this association will be discussed. This evidence provides a rationale for examining the hypothesis that dietary interventions prevent the development of depression or reduce existing symptoms.

Chapter 3 (Study 1) is a systematic literature review published in the British Journal of Nutrition entitled “Is there an association between diet and depression in children and adolescents? A systematic review”. It critically evaluates the association between diet and depression in children and adolescents. The review identifies and examines methodological differences in both study design and key constructs, and then synthesises the findings to make recommendations for future studies on the effects of nutrition on adolescent mood. The review highlights the importance of

nutrition as a potential way to prevent or treat mental health. Gaps in the current literature, such as lack of studies exploring both clinical and community samples, longitudinal designs, clear constructs to define diet, and failure to account for important confounds that influence low mood and diet in adolescents, are discussed. This chapter explains the rationale for Study 2 reported in the next chapter.

Chapter 4 consists of Study 2 investigating the association between diet quality and depressive symptoms in a healthy sample of UK adolescents. Participants completed online measures; a gold standard measure of depression, the Mood and Feelings Questionnaire, and a Food Frequency Questionnaire to calculate the nutritional intake. The nutritional data was then used to calculate diet quality using Diet Quality Index-International (DQI-I). The results show that there was no significant difference between diet quality and depressive symptoms. However, consumption of fruits and vegetables in young adults was significantly lower than the daily recommended amount. This finding, in addition to the studies investigating fruits and vegetables consumption in relation to mental health, suggests an association between this food group and depression. This suggests that consumption of micronutrients present in fruits and vegetables were also not consumed in the recommended amount. This data supports the hypothesis that flavonoids, phytonutrient present in many fruits and vegetables, may also be consumed in insufficient quantities and may be associated with low mood.

Chapter 5 is an introduction to flavonoids describing their biochemical structure and various health benefits. The human research on the effects of flavonoids on health and cognition is critically evaluated. In addition to fruits and vegetables (containing flavonoids) consumption being associated with low mood, impairments in cognition are also linked with depression. The theoretical relationship between flavonoid and cognition, and cognition and depression are discussed in this chapter and provides a rationale to explore the indirect association of flavonoids to depression, leading to Study 3 reported in the following chapter.

Chapter 6 consists of Study 3 published in *Nutrients* entitled "Effects of Acute Blueberry Flavonoids on Mood in Children and Young Adults". Evidence from human and animal studies have shown the potential benefits of flavonoids from a variety of food sources on general health, most especially on cognition. Cognitive disturbances, such as difficulties with attention and concentrating, problem-solving, directing thoughts and behaviours, are common symptoms of depression. It is therefore plausible that the beneficial effects of flavonoids on cognition may

provide additional benefits in mood disorders. This study is a randomised, cross-over experiment comparing the effects of acute blueberry flavonoid consumption and a placebo on transient mood in healthy children and young adults. The results show that acute wild blueberry consumption increased self-reported positive mood but did not decrease negative mood in young adults and children. This improvement in transient positive mood may be beneficial in preventing dysphoria, i.e. sustained periods of low mood, which is a strong predictor of the onset of major depressive disorder. Potential mechanisms which may be involved in flavonoids' effect on mood are discussed.

Chapter 7 is an addendum to Study 3, discussing the effects of acute wild blueberry intervention on executive functioning in young adults.

Chapter 8 contains Study 4, a double blinded, placebo-controlled crossover study investigating the effects of acute wild blueberry flavonoid intervention on transient mood and cognition, in young people, including a sub-sample who had elevated symptoms of depression. This was a replication and extension of Study 3, with clear distinction in mood state between the two groups and the addition of variety of cognitive tasks assessing working memory and verbal fluency in addition to accuracy and response time. Participants in both groups were assigned to both interventions in a random order, mood and cognition were assessed before and two hours post intervention. Though we fail to show any significant effects of intervention on transient mood and cognition in both the groups, this may be due to methodological limitations of this study. Replicating the study in participants with elevated symptoms of depression may increase power to demonstrate the beneficial effects of flavonoids on mood and cognition.

Chapter 9 consists of Study 5, published in British Journal of Nutrition entitled "Effects of 4 weeks daily wild blueberry supplementation on depression in adolescents". In light of increasing evidence from the previous studies in this thesis, this randomised double-blind, placebo-controlled trial investigated the effects of daily wild blueberry supplementation for 4 weeks on transient and chronic mood in adolescents. It demonstrated the chronic effect of wild blueberry flavonoid consumption on depressive symptoms in a sample of healthy adolescents. Those who received the flavonoid drink reported a significant decrease in their symptoms compared to those who received the matched placebo. Results require replication in both at-risk and clinical populations; however, the current results provide a very promising rationale for exploring wild blueberry flavonoids' effects on mood.

Chapter 10 is an addendum to Study 5 discussing the effects of chronic wild blueberry intervention (4 weeks) on cognition including executive functioning, working memory and rumination.

Chapter 11 consists of a general discussion of the collective studies and the potential mechanisms of actions that may be involved. Limitations and rationale for further studies are also discussed.

Chapter 1: Introduction to Major Depressive Disorder

1.1 Introduction

The term ‘depression’ is sometimes used to describe a transient mood state with fluctuating and short-lived emotional responses to everyday stimuli. Using this description, ‘depression’ is a ubiquitous part of life, characterised by fluctuating goals, unavoidable challenges and setbacks which are unpleasant but manageable for most. Depression can also be used to refer to a much more complex and impairing experience that is persistent, lacks obvious connection to the environment, impervious to mood repair techniques and interferes with the ability to function in relationships to self, others and work [(1)]. Major Depressive Disorder (MDD), also known as clinical depression, is a serious medical condition that affects adults as well as adolescents and the paediatric population and is a growing public health concern. Adolescents are a high-risk group for the development of depression, with estimates of the cumulative prevalence of MDD before the age of 18 years ranging from 8% to 20% [(2)]. According the World Health Organisation (WHO 2017) over 300 million people, of all ages, experience depression at any one time and it is the leading cause of disability worldwide, contributing to the overall global burden of disease [(3)]. Depression is also the second leading cause of years lived with disability globally and the primary driver of disability in at least 26 countries. Women are at approximately twice the risk of developing depression than men [(4)]. Mild depression accounts for 70%, moderate depression for 20% and severe depression makes up 10% of all cases. According to NICE (2011), the incidence of depression within the UK adult population ranges from 3-6% and those requiring treatment are estimated to increase by 17% to 1.45 million in 2026 [(5)]. Depression is also common in those with chronic physical health problems like heart disease, cancer, diabetes, musculoskeletal, respiratory, or neurological disorders, making up 20% of this population. The cost to the UK economy attributed to depression was estimated to be about £7.5 billion and the cost to treat depression in the UK was estimated at £1.7 billion [(5,6)].

Approximately 2-6% of children and adolescents meet diagnostic criteria for a diagnosis of depression [(7,8)]. According to the National Institute of Mental Health, depression is highly prevalent during adolescence, with up to 12.5% of 12 to 17 year-olds experiencing a major depressive episode in a 12-month period [(8)]. A meta-analysis including 26 epidemiological studies of British children and adolescents born between 1965 and 1996 showed that the one-year prevalence of depression was between 4% and 5% [(9)]. It is the second leading cause of death in

people aged 15-29 via suicide, which contributes to the burden of suicide and has a direct and indirect impact on the quality and length of life [3,10]. In the general population, the prevalence of childhood and adolescent depression ranges from 0.4% to 8.3% [11-13].

Childhood and adolescence are particularly important developmental periods marked by building and understanding of healthy relationships, exploring interests, developing important skills, transitioning to further education and work [14,15]. Additionally, there are also significant biological changes occurring during this period [16]. These include physical development including growth and sexual maturation, neuropsychological processes such as increased emotional liability, sense of vulnerability and development of abstract thinking [17]. Rapid neural and cognitive development also occurs during this stage [18].

The development of executive functioning such as planning, organizing, focusing, problem solving, decision making, and moderating behaviour is a central element of adolescent cognitive development. This cognitive control is associated with the prefrontal cortex, maturation of which is a key focus of adolescent brain development [19].

Experiencing depression during childhood or adolescence has many detrimental effects including diminished self-esteem [20], family dysfunction [21], poor physical health [22], increased risk for substance abuse [23], disrupted parent-child attachment [24], conflicted and unsatisfying relationships [25], decline in academic performance [20], and increased risk of dropping out of school [12]. In addition to experiencing this plethora of problems, the risk for morbidity and mortality in these young individuals across the life span is significantly increased [26,27].

A review of epidemiology of adolescent suicide and suicidal behaviour found that mood disorders contribute substantially to the risk of suicide [28]. A prospective case control study reported that from the 73 adults who experienced adolescent onset MDD, 7.7% had mortality due to suicide and the 37 adults with no past or current psychiatric disorder had 0% mortality. Furthermore, of the adults with adolescent onset MDD, 50.7% attempted suicide [29]. Thus, adolescent onset MDD not only increases the risk of suicide during adolescence, but this elevated risk continues into adulthood. In addition to this, childhood depression is persistent and often leads to increased risk of relapse during adolescence and adulthood [30-32].

1.2 Symptoms of Depression

According to the Diagnostic and Statistical Manual of Mental Disorder (APA: DSM 5) criteria depression can be diagnosed if a person experiences at least five symptoms of depression such as anhedonia, sleep changes, and if these represent a clear change from their previous psychological state [33]. They must persist for at least 2 weeks and interfere with everyday functioning. Although these criteria improve the reliability and diagnosis of MDD, empirical support for the presence of the five symptoms and their two-week duration is limited. Several studies on subthreshold depression highlights the dimensional nature of depression, commonly characterised by a cluster of depressive symptoms not meeting the full criteria for MDD. Individuals experiencing this subclinical level of depression also experience substantial impairment in functioning and are at high risk of developing MDD in the future [34].

In adolescents, the core symptoms of depression are depressed or irritable mood and anhedonia (reduced positive affect). Anhedonia refers to a diminished or complete loss of feelings of pleasure and enjoyment. This is perceived by lack of engagement or failure to respond positively to enjoyable events. Reduced motivation and anticipation may also be core features of this symptom and there may be specific deficits in the concept of reward [34-37].

Other symptoms of depression include basic circadian and biological rhythms of energy and negative cognitions. This involves weight changes due to significant increase or decrease in appetite. These weight changes are defined as a change of more than 5% of body weight in a period of a month and cannot be attributed to purposeful dieting [38].

Apparent changes in sleep patterns may also be experienced including insomnia (loss of sleep) or hypersomnia (increase in sleep). Approximately three quarters of those who are depressed experience insomnia symptoms and about forty percent experience hypersomnia causing distress, both of which are strong risk factors for suicide [34]. Frequent loss of energy and fatigue is also a common symptom of depression, which may occur independent of sleep difficulties. Physical fatigue includes reduced activity, tiredness, and increased effort with low physical endurance tasks. This may be accompanied with psychomotor retardation resulting in slowing of general motor processes which include speech, reaction times and information processing speed that is noticeable to others [34].

Cognitive processes are also impaired in most people with a diagnosis of major depressive disorder [39,40]. It is associated with deficits in cognitive functioning, especially in executive

functioning, working memory and processing speed [41,42]. Executive functioning refers to the ability to plan, problem solve and inhibit responses. A recent and comprehensive review concluded that depressed participants showed deficits across several aspects of executive functioning such as updating shifting and inhibition [43].

Depressed individuals may also express difficulties with working, visual and short-term memory with studies reporting decreased ability to retain new information [43-45]. Depressed individuals also show difficulty with cognitive flexibility, preventing them from dealing with life events and therefore prolonging the depressed state [46]. Individuals may also experience deterioration of their self-concept, ruminating about feelings of worthlessness or inappropriate and excessive amounts of guilt. These result in negatively judging and criticising oneself, feelings of inadequacy and thoughts such as one contributes nothing to others or the world in general. These negative thought processes often lead to self-harm or engagement in suicidal acts.

1.3 Treatment for depression

1.3.1. Adults: The most recent National Institute of Health and Care Excellence guidelines (NICE 2018) [47] identify that moderate to severe depression in adults should be treated with psychological therapy i.e. individual Cognitive Behaviour Therapy (CBT), Behavioural Activation (BA) or Interpersonal Therapy (IPT).

Cognitive Behaviour Therapy targets negative cognitions such as dysfunctional thoughts and core beliefs, in addition to information processing biases [48]. CBT is a collaborative therapy where the patient and therapist share knowledge and goals to help the patient solve their difficulties for themselves [48].

Behavioural Activation (BA) is a component of CBT, but as a treatment it uses learning theory to identify the key reinforcers that maintain depression. The core model underlying this therapy proposes that initial withdrawal from people and activities results in reduced positive reinforcement and low mood. This in turn increases avoidance, creating a cycle which then becomes difficult to break. BA identifies these behaviours and focuses on increasing reinforcement to improve mood. There is no difference in the effectiveness of BA and CBT [49,50] and therefore BA is also a recommended treatment for adults. However, BA is not recommended for use in adolescents as it has not been widely used or evaluated with this population.

Interpersonal Therapy (IPT) includes principles that are part of both psychodynamic psychotherapy and CBT. It combines the problem-solving element of CBT with focus on the interactions between depressive symptoms, interpersonal difficulties, and the ways in which they are mutually reinforcing. Methods to improve interpersonal functioning and tools for modelling external relationships are explored using this psychological therapy.

Pharmacological treatment may also be recommended on its own or in combination with psychological therapy. Selective Serotonin Reuptake Inhibitors (SSRI) is the first drugs of choice for adult depression. These include drugs such as fluoxetine, fluvoxamine paroxetine, sertraline, and citalopram [(51)]. These pharmacological agents act on serotonergic neurons by inhibiting the uptake of serotonin into the nerve terminals. Serotonin is one of the neurotransmitters involved in mood regulation. Serotonergic pathways are present in several areas of the brain including the frontal cortex, striatum, thalamus, hypothalamus, hippocampus, and amygdala. Serotonin released into the synapses of these areas act on a multitude of postsynaptic serotonin receptors. Serotonin also interacts with presynaptic serotonin receptors and serotonin receptors on a cell body, inhibiting the further release of serotonin. SSRIs block the reuptake of the serotonin into the presynaptic nerve thus increasing the synaptic concentration of serotonin thereby increasing positive mood [(52)].

Mirtazapine is another class of antidepressant and is associated with the enhancement of serotonergic and noradrenergic systems in the central nervous system. It blocks the inhibitory presynaptic α_2 -auto receptors leading to enhanced availability of the neurotransmitter norepinephrine (also involved in mood regulation) and therefore an enhanced release of norepinephrine in the synaptic cleft. It also antagonises receptors in the serotonergic nerve terminals resulting in enhancement in serotonin release [(53)].

If SSRI is not effective or suitable then tricyclic antidepressants (TCA) Iofepramine and/or Nortiptyline may be offered. Tricyclic antidepressants (TCAs) are first generation medication for depression. They target both serotonergic and noradrenergic pathways, enhancing the concentrations of both norepinephrine and serotonin. Furthermore, they block the action of acetylcholine, increased levels of which decreases manic symptoms but also induces severe depression [(54)]. Resorting the balance of these neurotransmitters results in alleviation of depressive symptoms. However, the adverse side effects, such as sedation, postural hypotension, blurred vision, dry mouth, and constipation resulted in TCA not being the first choice of treatment in depressive disorders.

1.3.2 Young people: Only one pharmacological agent, Fluoxetine, is recommended for treatment of young people with depression. The most recent National Institute of Health and Care Excellence guideline, (NICE 2019) recommends psychological therapies, CBT, IPT and family therapy [55,56]. At the present time, there are no antidepressants that have UK marketing authorisation in adolescents. However, the guidelines suggest considering combining psychotherapy with the SSRI fluoxetine. A systematic review of unpublished and published SSRI trials reported that the published data suggested favourable risk/benefit profiles for some SSRI drugs [57]. However, when unpublished data was taken into consideration, the risk/benefit ratio shifted towards unfavourable. Similarly, CBT is a better validated treatment for adult depression [56] and is known to reduce symptoms and relapse [58-60]. However, trials investigating its effectiveness on the adolescent population have not produced convincing conclusions [61,62]. A meta-analysis reviewed five randomised control trials comparing the effectiveness of SSRI alone and in combination with CBT in young people suffering from depression [63]. No evidence of significant benefit of combination of medication and CBT on depressive symptoms or suicidal ideations were observed after treatment or at follow up. Not many trials exploring the effects of IPT in adolescents exist. Only two trials have compared IPT to CBT in depressed adolescents [64,65]. The effectiveness of 12 weeks of IPT and CBT in 71 adolescents (13-17 years) against a control group (adolescents on a waiting list) was compared [64]. IPT and CBT significantly reduced participants' self-reported symptoms of depression when compared to the control, but no significant difference was observed between the IPT and CBT. Similarly, another trial compared group and individual IPT and CBT in 112 young people (12-18 years) and found no significant difference between the group and individual IPT and CBT. However, a significant difference in self-reported depression was observed between those who underwent CBT and IPT, compared with those in the CBT group reporting greater reduction in their depressive symptoms [65].

Given the limited evidence on the effectiveness of the existing treatment for young people, mental health services are under extreme pressure to target resources for treatment of those with severe disorders and up to 50% of such referrals would benefit from early detection and intervention. Therefore, there is a pressing need for an alternative support for children and adolescents suffering with mental health difficulties. It is equally important to investigate ways by which to prevent this onset of depression especially in young people.

1.4 Aims of the Thesis

This thesis will explore the association between nutrition and depression as a possible preventative and treatment strategy for depression. Given the prevalence and long-term impact of depression on adolescents, further work is urgently needed to understand ways in which depression in adolescents can be targeted. Existing research investigating the effects of various macro and micronutrients including proteins, fats, vitamins, and minerals, individually and in combination as part of an overall diet will be explored. Additionally, association between overall diet quality and depressive symptoms will also be investigated in a community sample of UK adolescents. Intake of fruits and vegetables by adolescents to daily recommended intake will also be compared. The presence of flavonoids in these fruits and vegetables are of great interest. Flavonoids have shown to have numerous health benefits but their effects on mood are limited, especially in young adults. This thesis then proceeds to investigate the effects of acute and chronic blueberry intervention on mood and cognition in young adults.

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Chapter 2. Association Between Nutrients and Depression

2.1 Introduction

The relationship between mechanisms that link nutrition and physical health are widely researched and relatively well understood, especially when compared to our exploration and understanding of the relationship between nutrition and mental health. Mental health disorders, such as depression, are typically described as biochemically based phenomena requiring antidepressant medications, or as psychological phenomena that require treatment via counselling or therapy. However, within the past decade, there has been a rising interest in how food and nutrition may play a role in mental health. Food patterns, such as poor appetite, skipping meals and dominant desire for sugary food, are noticeable not only during episodes of depression but also preceding it. Often, several nutrients, such as essential vitamins, minerals, amino acids and unsaturated fatty acids, are found to be deficient in those suffering from depression and daily supplementation of these nutrients have shown to be effective in reducing the depressive symptoms [(1)]. As depression is a biochemical imbalance, it is important to explore how the brain normalises biochemistry during low mood by using nutrition to exert beneficial effects on modulating or correcting these biochemical imbalances. There have been several studies indicating associations between nutrition and depression in adults. Even though the exact cause and effect relationship between nutrition and depression is not fully understood, many research studies have shown links between specific nutrients and overall diet with mood. In this chapter, multiple nutrients that may be deficient and the mechanism by which they influence the development, maintenance and relapse of depression are discussed.

2.2 Polyunsaturated Fatty Acids (PUFA)

Polyunsaturated Fatty Acids (PUFA) are fatty acids that can be classified according to their chemical structure as omega-3 or omega-6 fatty acids. Omega-3 fatty acid is synthesised by alpha-linolenic acid to form the more complex omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [(2)]. Omega-6 fatty acids are derived from linoleic acid (LA), gamma-linolenic acid (GLA), arachidonic acid (AA) and dihomo-gamma-linolenic acid (DGLA). LA and GLA are both essential fatty acids as human bodies are unable to synthesise these from simpler precursors. These fatty acids can be naturally found in oily fish, walnuts,

flaxseed oil and soybeans. The importance of omega-3 and omega-6 PUFAs in the pathogenesis and management of depressive disorders has increased in the light of recent research.

Epidemiological studies have demonstrated that there is an inverse relationship between the prevalence of depression and the amount of fish consumed [2-4]. It has also been shown that depression is common in individuals with low concentrations of omega-3 PUFA and DHA in the red blood cells, thus reflecting levels of fatty acid composition in the brain [5-7]. Some studies have shown an inverse relationship between omega-3 fatty acid levels and severity of depression [6-8]. A study compared the rates of depression in nine countries with estimated fish consumption and reported an inversely proportional relationship, whereas western countries had an annual prevalence of depression ranged from 3-6% and a low to moderate fish intake (11-32 kgs). On the other hand, countries with higher annual fish consumption, such as Japan, had a depression rate of 0.12% [9]. In areas such as Greenland, diets predominantly consist of cold-water fish that are uniquely rich in long chain omega-3 PUFA, EPA and DHA. Depression related incidents in these areas are low to none despite the extreme climate and challenging environmental conditions [10].

Furthermore, decreased consumption of omega-3 fatty acids and increased intake of omega-6 fatty acids have been correlated with an increase in mental health disorders [11]. A number of small clinical studies observed that levels of omega-6 in the tissues of depressed patients were high when compared with healthy individuals [5,8]. This decreased ratio of omega-3 and omega-6 fatty acids in the erythrocytes and plasma have also been associated with severity of depression [5-8]. Similarly, self-harm has also been found to be more common in individuals with lower intakes of essential fatty acids [4,12,13].

There are several theories with regards to how omega-3 fatty acids may contribute to the prevention and treatment of depression. One of the core pathophysiological processes underlying depression is the imbalance of serotonergic and noradrenergic neurotransmission. Reduced uptake of serotonin is shown to be one of the underlying factors contributing to low mood in depressed individuals [14]. Furthermore, the metabolite 5-hydroxyindoleacetic acid (5-HIAA), that reflects serotonin turnover has been reported to be decreased in several psychiatric conditions including violent suicide attempts during depression [15]. Omega-3 fatty acids are suggested to influence membrane fluidity of several types of cells, including brain and neuronal [16]. Membrane fluidity is defined as the fatty acid chains comprising of the lipid bilayer microstructure of the cell membrane [17]. In optimal state, neuronal membranes are involved in secretion of neurotransmitters, effective neurotransmitter binding, intercellular signalling,

production of secondary messengers, ion channel functioning, receptor functioning, enzyme activity and gene expression. Omega-3 PUFA are essential components of lipid bilayer in such membranes and deficiency adversely affects the signalling pathways in neurons. A growing body of evidence suggests that membrane lipid abnormalities occur in depression and that omega-3 PUFA, in particular DHA, is depleted in depressed subjects [5-8,17,18].

Additionally, omega-3 fatty acid is also shown to regulate signal transduction by enhancing G-protein mediated signal transduction [19], membrane bound enzymes [20] and protein kinase C, all of which are located on the cell membrane [21]. These membrane changes result in increased transport of dopaminergic and serotonergic neurotransmission which, in turn, elevate mood [22]. Additionally, PUFA is also known to influence numbers and function of serotonin (5-HT) and dopamine receptors (DR-2). On the other hand, omega-3 deficiency results in 5-HT receptor density increasing in the frontal cortex, thought to be an adaptation to decreased serotoninergic function, thus resulting in deterioration of mood [23].

High levels of cortisol in the blood due to the hyperactivity of the Hypothalamus-Pituitary-Adrenal (HPA) axis has also been associated with depression [24]. It has been speculated that EPA may be involved in the regulation of HPA dysfunction by reducing corticotrophin releasing factor expression and corticosterone secretion [25]. Additionally, omega-3 PUFA is thought to inhibit the transport proteins responsible for the increase of cortisol transport through the blood brain barrier [26-29].

2.3 Proteins

Proteins are important building blocks made up of amino acids. The human body requires twenty amino acids, twelve of which are manufactured by the body and eight of which must be obtained through diet, making them essential. A high-quality protein diet contains meats, milk, other dairy products, egg, and plant proteins such as beans, peas, and grains. Plant proteins however, lack in a few essential amino acids. Amino acids, and in turn protein intake, have been related to brain function and mental health. Amino acids, such as tyrosine and tryptophan, are essential precursors of neurotransmitters that are associated with low mood and aggression (dopamine and serotonin respectively) [30]. Evidence suggests that tryptophan availability to the brain directly influences the serotonin levels [31]. There is also abundant evidence demonstrating the importance of serotonin in disorders such as depression. Experimental studies show that depletion of

tryptophan, precursor of serotonin, induces symptoms of depression in vulnerable individuals (i.e. patients in remission or their family members) but not in healthy individuals [32]. On the other hand, administration of tryptophan has been shown to increase serotonin synthesis in both rats and humans, this increase being large enough to influence behaviour and mood [33]. The effects of readily available tryptophan through meals or either supplementation has been shown to change sleep and mood patterns [34,35]. Overall, although there is insufficient evidence to support therapeutic use of tryptophan and 5-HTP supplements in depression [36], there is enough evidence to recommend the consumption of adequate protein intake following healthy eating guidelines.

2.4 Carbohydrates

Carbohydrates are naturally occurring polysaccharides that play an important role in the structure and function of human beings. It is thought that carbohydrates can affect mood and behaviours in humans. Food with a low glycaemic index, such as whole grains, fruits, and vegetables, are known to provide a moderate but lasting effect on brain chemistry, mood, and energy level. Foods with high glycaemic index, like sweets, tend to improve mood immediately but the effects are temporary [37]. Carbohydrate rich meals trigger the release of insulin which helps the distribution of sugar into cells to be converted into energy, simultaneously triggering the entry of tryptophan to the brain. Even though tryptophan, being an amino acid, is present in protein rich foods, it is hypothesised that having foods rich with carbohydrates is more effective in terms of levels of tryptophan in the brain and subsequently serotonin levels. It is suggested that the carbohydrate to protein ratio in meals has an influence on the ratio of tryptophan and other large neutral amino acids (LNAAs) which includes tyrosine, phenylalanine, leucine, isoleucine, and valine. LNAAs are known to compete with tryptophan for blood brain barrier transport and several studies have shown a decrease in plasma tryptophan to LNAA ratio after consuming a protein or protein and fat rich meal [38].

On the contrary, carbohydrates, especially those with high glycaemic index, have been associated with an increase in plasma tryptophan-LNAA ratio [39]. Dietary carbohydrates, due to its ability to promote the secretion of insulin, have the ability to modify plasma amino acid patterns which enhance the uptake of circulating tryptophan into the brain [40]. Insulin has little or no effect on plasma tryptophan levels, free or albumin bound (both accessible to the brain). Instead, it lowers the levels of LNAA by promoting their uptake in the muscles, resulting in higher levels of

tryptophan and less competition of its uptake through the blood brain barrier. However, carbohydrate rich and protein poor diets are not healthy on a day-to-day basis and even small amounts of protein blocks the rise of tryptophan [41]. The ratio of amino acids in the plasma favours tryptophan only when the protein intake offers less than 2% of the total calories. However, as little as 5% of the calories in the form of protein is enough to ensure that this does not happen. No normal meal will contain so little amount of protein, including foods that are considered rich in carbohydrates. For example, 10% of the calories in potatoes are obtained from protein and 15% in bread.

Some studies agree that carbohydrate consumption can improve mood or relieve depression, however, not through nutritional properties but by psychological mechanisms [42]. Due to carbohydrate rich foods being more palatable, they release endorphins that affect mood. Overall, it is difficult to interpret the results from these studies as they are correlational in nature, thus making it difficult to tell whether carbohydrates lead to a better mood or those who are depressed eat or crave more carbohydrates.

2.5 Vitamins

2.5.1 Folic acid: Depression has also been associated with low levels of some vitamins and minerals. Levels of folic acid (vitamin B9), which is present naturally in a variety of foods, is the most researched vitamin with regards to depression. It has been suggested that low folic acid levels are associated with depressive symptoms [42-45]. Low levels of folic acid have also been shown to predict a poor response to antidepressants [46] and several studies show that depressed individuals who do not respond to conventional antidepressant treatments were deficient in folic acid [47-49]. A controlled study reported an enhance in antidepressant medication effectiveness after 500mcg of folic acid supplementation was administered [50]. Folic acid has a critical role in metabolic pathways in the brain, deficiency of which has been the most common neuropsychiatric manifestation in depression [51]. Firstly, it plays an important part in metabolism of homocysteine (a common amino acid in the blood), accumulation of which is associated with depression [52]. Studies have shown that at least 20% to 30% of depressed patients show high levels of homocysteine [53-54]. Secondly, low levels of monoamines, a result of folate deficiency, are also known to contribute to the development of depression [47]. Monoamines are neurotransmitters dopamine, noradrenaline, and serotonin, all of which are involved in mood regulation. Additionally, a number of studies suggest that the ability of folic acid to act as an

antidepressant is due to its ability to act as an SSRI [55]. It is still unclear whether poor nutrition, as a depressive symptom, causes folate deficiency or primary folate deficiency results in depression.

2.5.2 Vitamin B12: Is present in fortified foods, such as cereals and soy products, and in fish and beef liver. It is known to play an essential role in DNA synthesis and neurological function [56]. Deficiency of B12 is associated with haematological, neurological, and psychiatric manifestations such as irritability, personality changes and depression [57]. Vitamin B12 has been shown to be decreased in depressed individuals when compared to healthy ones [58,59]. Furthermore, it has been reported that individuals with vitamin B12 deficiency are 70% more likely to develop depression [60]. Deficiency of vitamin B12 is harder to detect as it is usually masked by the availability of folic acid. Similar to folic acid, deficiency in vitamin B12 contributes to increased levels of homocysteine which, in turn, contributes to depression [61]. Another theorised mechanism by which vitamin B12 may play a role in depression is due to its involvement in one carbon metabolism, which, in turn, exerts an important function in the regulation of mood [62].

2.5.3 Vitamin B3: Also known as niacin/nicotinic acid and nicotinamide/niacinamide, is an essential nutrient obtained from diets consisting of a variety of vegetables, tuna, whole and processed foods. It is one of the vitamins required for optimal brain functioning, increase in cerebral blood flow and neurotransmitter synthesis, some of which are involved in mood regulation [63]. A few controlled clinical trials have shown beneficial effects of vitamin B3 on depression, showing that it may have anti-depressive effects [64]. Though limited evidence shows a significant change in depression with vitamin B3 supplementation alone, it has been shown to be effective in treating depression in combination with tryptophan [64].

2.5.4 Vitamin B6: The richest sources of vitamin B6 are fish, beef liver, fruits, potatoes, and other starchy vegetables. Vitamin B6 is essential for normal central nervous system and brain functions involved in modulating mood through several pathways. It is a cofactor for enzymes that synthesise the neurotransmitters, serotonin, epinephrine, norepinephrine, and Gamma Amino Butyric Acids (GABA) [65]. In addition to this, low levels of B6 also result in elevated levels of homocysteine which is associated with depression. Studies show vitamin B6 is negatively associated with internalising behaviours such as depression and anxiety. A study in Japan showed adolescents consuming a non-western diet and the resulting higher intake of B6 showed reduced

depressive symptoms [66]. Similarly, studies in older age groups reported those who were depressed had low levels of vitamin B6 [67,68].

2.6 Minerals

2.6.1 Zinc: Is a micronutrient and is the second most abundant transition metal present in all body tissues. It is widely distributed in the central nervous system [69-71]. Its concentrations are highest in the hippocampus and amygdala [72]. Zinc is essential for optimal function of the brain and neural structures [73-76]. Low levels of zinc in the plasma is associated with low mood when compared with healthy adults [77]. Another study shows that serum zinc levels are significantly lower in people with major depression compared to healthy adults and its correlated with severity of depression [78,79]. Furthermore, it is also reported that 25mg of zinc for 12 weeks improved the effectiveness of antidepressant treatment in patients who were previously resistant to antidepressant treatments [80]. A review concluded that the existing evidence suggests potential benefits of zinc supplementation as a standalone intervention or as an adjunct to a conventional intervention or therapy for depression, even though the number of high-quality trials investigating the effects of zinc supplementation on depressive symptoms is limited [81]. Mechanisms by which zinc plays a role in depression are hypothesised to be through modulating inflammation, neuroplasticity, neurogenesis, and oxidative stress [82] and changes in any of these could contribute to the pathology of depression [83]. However, the number and qualities of these studies are limited. Additionally, decreased levels of zinc indirectly contribute to inflammation as this decreased level may lead to depletion of omega-3 PUFA, further adding to the symptomatology of depression [84].

2.6.2 Selenium: Is another micronutrient essential for humans, though high doses can be toxic [85]. Selenium is found in foods such as liver, kidney, beef, fish, eggs, Brazil nuts, fruits, and vegetables. Studies on selenium deprivation show that a low intake of selenium is negatively correlated with low mood in healthy adults [86]. It was also observed that a selenium poor diet resulted in deterioration of mood, whereas an increase of its quantity in diet improved mood [87]. Studies show that increasing dietary selenium can improve mood in healthy men [87], whereas, low intake is correlated to increased risk of developing a new depressive episode [88]. Furthermore, it has been shown that selenium supplementation during pregnancy was effective in the prevention of postpartum depression [89]. Selenium supplementation (100 μg/day) in healthy individuals over 5 weeks resulted in improvement in depressive symptoms, especially in those

with poor dietary selenium intakes [90], though larger randomised controlled trials in elderly women showed no such effect. Selenium is proposed to improve mood by its anti-inflammatory and antioxidant functions in addition to being extremely important for brain function [85, 91-92]. It also has a significant modulatory effect on dopamine which is involved in pathophysiology of depression [93,94]. Though implications of selenium intake on depression warrants further investigation in different populations, the existing evidence suggests that selenium has the potential to be a factor in prevention and management of depression.

2.6.3 Magnesium: Another essential mineral in the human body is magnesium. Magnesium acts as a cofactor for over 300 enzymes and is available in several food items. Literature suggests that magnesium is connected with brain biochemistry in addition to fluidity of neuronal membrane [95,96]. Like vitamins and PUFAs, studies show an inverse association between magnesium consumption and depression [97,98]. Cerebral spinal fluid magnesium concentration is reported to be low in patients with history of suicidal behaviour [99,100]. Individuals suffering from depression also have lower plasma/serum levels of magnesium when compared to healthy individuals [101-103]. Some studies suggest that patients who do not respond to anti-depressive treatments have lower serum magnesium levels than patients who do respond [104]. Human studies propose that low magnesium is associated to inflammatory and oxidative stress, which in turn is associated with depression [105]. It is also suggested that insufficient long-term magnesium intake can result in development of systematic inflammation which, in turn, may heighten the symptoms of depression [106-109].

2.6.4 Chromium: Is a trace element that occurs in nine oxidation state, some of which are essential to humans while others are highly toxic [110-111]. Main sources include broccoli, Brewer's yeast, beef, eggs, liver, oysters, and chicken. The few human studies carried out regarding chromium and depression suggests that intake of chromium (600mcg/day) for at least 8 weeks helps reduce depressive symptoms, such as increased appetite, carbohydrate cravings and fluctuations in mood [112]. Clinical studies have confirmed the antidepressant properties of chromium in mood disorders [113]. This has shown to be effective in atypical depression, which is hypothesised to be related to down regulation of serotonin receptors [114]. It has also been shown to reduce symptoms in premenstrual dysphoric disorder and dysthymic disorder [115,116]. It is suggested that chromium has this effect due to its role in serotonin receptor modulation, monoamine neurotransmitter system, insulin sensitivity and central nervous system functions, such as cognition and mood [114, 117-120].

2.7 Discussion

Nutritional status plays a crucial role in mental health and poor nutrition may contribute to pathogenesis of depression. Evidence supports the relationship between intake of several different nutrients and mood and that adjunctive uses of some nutrients could improve the outcome of depression. However, there is insufficient evidence to support routine supplementation and due to the studies not including larger populations, being of short duration and of generally poor design, this evidence is far from robust. Additionally, it is unclear how to develop treatments using these nutrients that are target specific, as the biological mechanisms underlying these relationships remain poorly understood.

There is a strong possibility that these nutrients could be used effectively in prevention and treatment of depression exclusively or in combination with other nutrients or existing treatments. Although some of the nutrients, such as dietary antioxidants and a few trace elements, have not been investigated rigorously, the data still suggests a biological plausibility in affecting brain function and modulating mood. Although the nutrients discussed here have different physiological roles in the maintenance of mental health, the common factor is that deficiency in any is very common in those suffering from depression. The direction of this association remains unclear. Whether having a poor diet leads to depression or vice versa, hence resulting in nutritional deficiency, warrants further investigation. The current epidemiological, correlational, and clinical studies suggest this relationship is multifaceted and bidirectional. However, literature is limited in the amount of longitudinal studies investigating the direction of this relationship.

It is still important to consider the methodological limitations of these studies from heterogeneity, lack of randomisation and/or blinding, low statistical power due to small sample sizes and insufficient duration of exposure. In addition to this, there is inconsistency in the literature with regards to the inclusion criteria of participants, in terms of age, gender and other diseases, resulting in no representative samples. Another important factor that is commonly overlooked is the participants' ability to absorb these nutrients and any allergy and intolerance participants may have. Therefore, more high quality, rigorous clinical trials (taking into account wider range of nutrients) are required to understand what agents are useful in what group, under which conditions and the resulting outcome. Additional challenges include replication in both clinical and community populations across all ages, population level dietary interventions, identifying the biological pathways by which these changes in mood are mediated, examining their effects on other factors that are associated with low mood (such as gut microbiome profiles, base line

nutritional and inflammatory status), lifestyle and other dietary behaviours. Overall, the factors contributing to the development and pathology of depression are complex and multitudinous. Therefore, dietary change should be considered as a part of a range of lifestyle strategies, such as exercise and cessing smoking, which supports mental health in addition to, not necessarily instead of, other existing pharmacological and psychological interventions. As nutrients are not consumed in isolation, studies examining overall patterns will be able to provide a more realistic picture and will be more easily translated to public health advice. The next chapter therefore discusses the more recent research that has moved away from examining specific nutrients to overall dietary patterns. In conclusion, there is enough evidence to suggest further investigation in clinical settings as to promote the benefits of dietary supplementation and improvement in those with major depressive disorders.

2.8 References

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Chapter 3. Study 1: Is there an association between diet and depression in children and adolescents? A systematic review

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Is there an association between diet and depression in children and adolescents?

A systematic review

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Abstract

This review critically evaluates previous research investigating the association between dietary intake of young people and depression and related mental health problems. A systematic literature search was conducted using electronic databases such as PSYCINFO, MEDLINE, PUBMED and COCHRANE. Twenty studies were identified that met the inclusion criteria and were subsequently rated for quality. The studies used a range of methods to measure dietary intake and mental health. Important potential confounding variables (e.g. socio-economic status) were often not included or controlled. There were also inconsistencies in the use of key constructs, which made comparisons between studies difficult. Despite some contradictory results, overall, there was support for an association between healthy dietary patterns or consumption of a high-quality diet and lower levels of depression or better mental health. Similarly, there was a relationship between unhealthy diet and consumption of low-quality diet and depression or poor mental health. However, where significant relationships were reported effect sizes were small. Future research on the relationship between diet and mental health in young people should use more clearly defined constructs to define diet and include or control for important confounds.

3.1 Introduction

In any given year, approximately 20% of children and adolescents globally have mental health difficulties, including major depressive disorder. Depression has been ranked as the second most common cause of death in adolescents, via suicide [1, 2]. As mental health problems often start in childhood or adolescence, they are strongly associated with other developmental and health conditions affecting quality of life, social, academic performance, personality disorders and substance abuse in adult life [3-6]. The recommended treatment for this age group is limited and includes psychological therapy which may be combined with one pharmacological treatment, Fluoxetine [7]. Both of these treatments, however, are only moderately effective, with up to 50% of young people not responding to treatment or experiencing relapse and further episodes of depression [8-10]. An important area for development therefore is to prevent mental health problems via public health interventions that can be delivered to a whole population of children and adolescents.

Over the past decade, a number of studies have suggested that diet could play an important role in treatment and prevention of depression. Two main approaches have been used to examine this relationship. A number of studies have investigated the impact of individual nutrients such as omega-3 fatty acids [11, 12], vitamins such as B12 [13] and minerals such as zinc, selenium and iron [14-16]. Additionally, several intervention studies have examined the effect of supplements containing more than one nutrient (for example multivitamins, eicosapentaenoic acid, and docosahexaenoic acid) on mood [17-19]. However, the idea of investigating individual nutrients to ascertain whether that single ingredient is responsible for improving mood is problematic. Mood regulation is influenced by a number of different neurochemical pathways (e.g. serotonin and dopamine), with each requiring several nutrients to supply the metabolites necessary for production of the individual neurotransmitters involved in regulation of mood [20].

An alternative approach has been to explore the effects of the whole diet and eating patterns on mood. In correlational epidemiological studies of adults, an ‘Unhealthy’ and ‘Westernised’ diet was associated with an increased likelihood of mental disorders and psychiatric distress [21-24], whilst a ‘Healthy’ or ‘good’ quality diet was associated with better mental health [21, 25-28]. However, a number of other factors such as socioeconomic status (SES), household income and educational levels also influence dietary choice and thus need to be included as potential confounds [29,30].

Overall, studies with adults that have investigated the relationship between diet and mental health suggest that the relationship is complex and potentially, bidirectional [31]. Given the development of the brain during childhood and adolescence, and the emergence of depression during adolescence, the impact of diet on mental health may plausibly be greater during this period than later in life [3,4.32]. Additionally, as children age into adolescents they become more independent in the type and choices of food they consume and develop preferences towards junk and fast foods which further influences the food choices made during adulthood [33]. Therefore, the relationship between diet and mental health in young people and children therefore warrants specific attention.

A recent review identified and reviewed 12 epidemiological studies that examined the association between diet and mental health in young people [34]. They concluded that there was evidence for a significant relationship between an unhealthy diet and worsening mental health. Our review aims to advance knowledge in this field by (1) using a more sensitive measure of assessing methodological quality, and (2) assessing effect sizes across studies so that data can be compared on a single metric. Together, this will help describe the current status of the field, identify key methodological challenges facing researchers, synthesise and integrate existing research to highlight future research opportunities and implications for the development of dietary strategies to prevent childhood and adolescent depression.

3.2 Methods

3.2.1 Search strategy: A systematic literature search was conducted of social sciences, medical, health and psychiatric databases (i.e. PSYCINFO, MEDLINE, PUBMED, BIOSIS COCHRANE LIBRARY and SCIENCE DIRECT). We identified relevant literature, published in the English language, from 1970 up to April 2016. Reference lists of related studies and reviews were also searched.

The search was carried out using the following combinations of key terms: internalising disorders or internali* or mental health or depression or depr* or depressive disorders or anxiety or anxi*or anxiety disorders or affective disorders or mood or mood disorders or wellbeing **AND** diet or nutrition or diet quality or dietary patterns **AND** youth or young people or adolescents or adol*or children or teen. As anxiety disorders commonly co-occur in children and adolescent with depression, anxiety having an earlier age of onset, they were also included in the literature search

[35-39]. However, diet and its relationship with depressive disorders is the primary objective of this review.

3.2.2 Inclusion criteria:

Studies eligible to be included in this review were:

1. In English language
2. Available as full text (including abstracts of meeting etc.)
3. Included children and young people 18 years and younger in the sample.
4. Study designs were case control, cross sectional, epidemiological cohort, experimental trials.
5. Examined the association between nutrition, dietary pattern, diet quality and internalising disorders (including low mood, depressive or anxiety symptoms and emotional problems).
6. Diet or nutritional intake measured via self-report (food frequency questionnaires, diet records) or controlled weighed food records, observation or use of biological markers.
7. Diet quality measured by calculating scores from food frequency data or diet quality and diet patterns defined as overall habitual dietary intake.
8. Internalising disorders measured using self-report, doctor's diagnosis, medical records, interview, or depression/anxiety rating scales.

3.2.3 Exclusion criteria:

Exclusion criteria were as follows:

1. Studies focused on disorders of eating or dietary restraint for weight loss purposes
2. Reported internalising disorder as a secondary problem to physical health problems e.g. diabetes and heart disease
3. Studies using only pregnant women as participants.
4. Animal studies
5. Studies that focused on individual nutrients specifically.
6. Studies where all participants were over 18 years
7. Mental health data limited to measures of behaviour or conduct (externalising problems)

The methodological quality of each study was assessed independently by S.K and S.R, using the National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort and

Cross-Sectional Studies. Disagreements were discussed with C.W and a shared rating was given. Methodological criteria evaluated included: (a) bias in selection of participants, measurement, or information with high risk of bias translating to a rating of poor quality and (b) study designs that could help determine a causal relationship between diet and mental health (Table 1).

Table 1: NIH Criteria list for assessing study quality

	Criteria list
1	Was the research question or objective in this paper clearly stated?
2	Was the study population clearly specified and defined?
3	Was the participation rate of eligible persons at least 50%?
4	Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were the inclusion and exclusion criteria for being in the study pre-specified and applied uniformly to all participants?
5	Was a sample size justification, power description, or variance and effect estimates provided?
6	For the analysis in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7	Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8	For exposure that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?
9	Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
10	Was the exposure(s) assessed more than once over time?
11	Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
12	Were the outcome assessors blinded to the exposure status of participants?
13	Was loss to follow up after base line 20% or less?
14	Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s).

3.3 Results

This section describes in detail the process of literature selection, quality ratings of the studies, methodology used and also a summary of results of these studies.

***3.3.1 Selection*:** A total of 3,014 studies were identified as a result of the initial search. Further screening identified 98 studies relating specifically to nutrition and mood. Of these, 78 were excluded, typically because participants were not in the appropriate age range, depression and/or anxiety was not measured, depression/anxiety were secondary to physical health problems, or studies included calorie restraint or binge eating.

Full details of screening, filtering and our selection process for the studies included in this review are shown in Figure 1. Twenty studies that met the inclusion and exclusion criteria were identified. Study populations were from United States, United Kingdom, Australia, Canada, Germany, Norway, Spain, Malaysia, Pakistan, Iran, and China. Even though the traditional diets of non-western countries may differ, most of these studies investigated the consumption of junk or Westernised foods. Two studies, one from China and another from Norway, examined both a Westernised and their traditional diets. Key features of the selected studies are presented in Table 2.

Data was extracted from 17 cross-sectional studies and 3 prospective cohort studies with follow up periods ranging from 2-4 years. No experimental studies or clinical trials were identified. The total number of participants recruited across the 20 studies was 110857, although, two studies used participants from the same data set (RAINE; with n=1324 and n=1598 respectively) [40, 41]; 109533 unique individual participants were recruited in total to these studies. There were 51834 males and 49588 females although some authors did not clearly state the number of boys and girls in their studies [42,43]. The age of participants ranged from 18 months to 18 years.

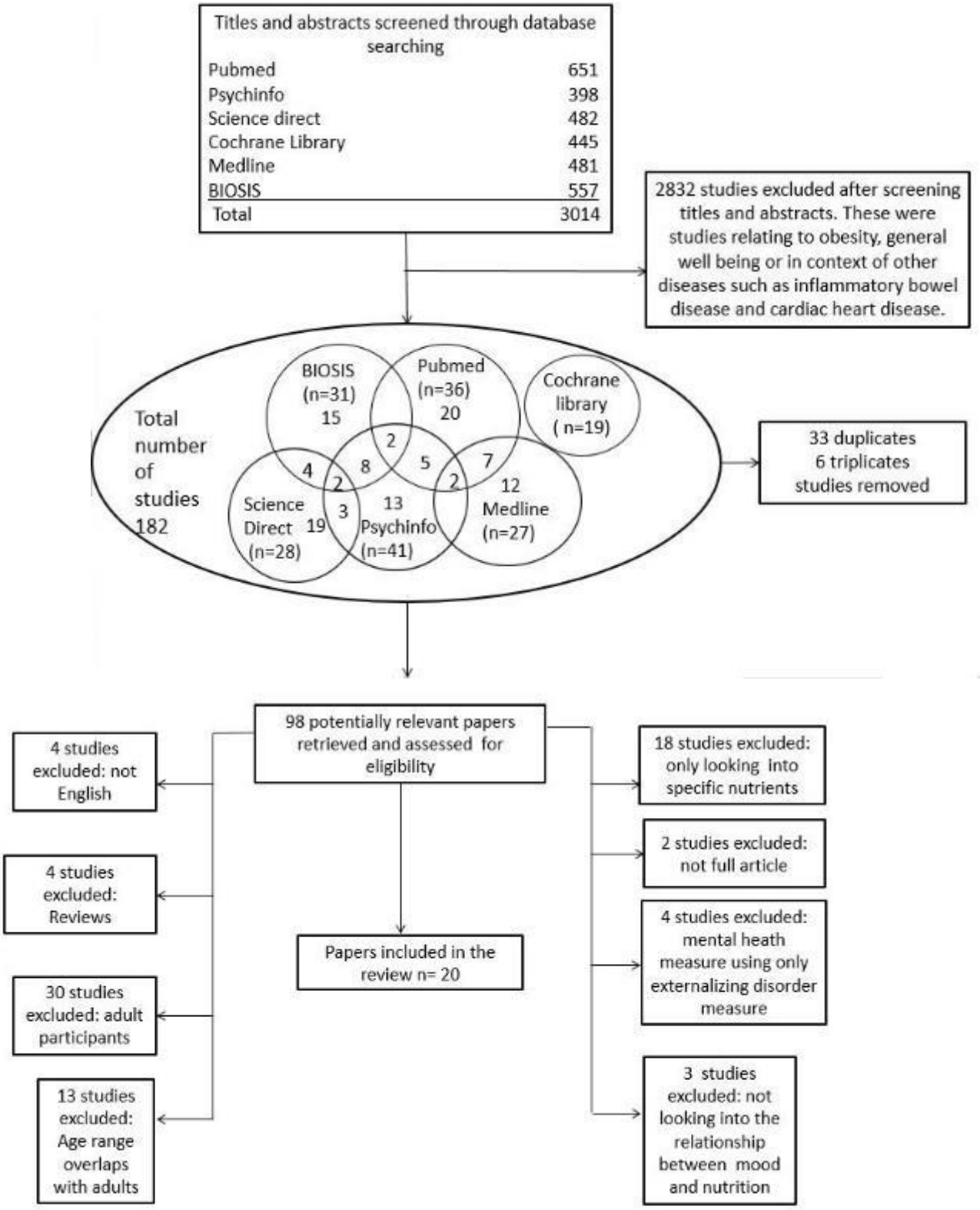

Figure 1. Showing the selection process of studies included in this review.

3.3.2 Overall quality: Using the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies, the quality of the majority of studies was rated as 'fair' (N=16), with three studies classified as 'good' [43-45] and one rated as 'poor'[42]. Key methodological features of each study are outlined in Table 2. Common methodological weaknesses included inadequate measurement of the key variables (diet and mental health) which will be discussed in more detail below.

3.3.3 Measures of diet: Several different measures were used to measure dietary/nutritional intake across these studies. The most common and relatively reliable methods used were food frequency questionnaires including Harvard Youth/ Adolescent Food Frequency Questionnaire (YAQ FFQ; a widely used validated questionnaire) and Commonwealth Scientific & Industrial Research Organisation FFQ (semi-validated for use in adults). Three-day food records, which are more reliable than FFQs, were used by one study [46]. Additionally, a single study calculated absolute food consumption (to nearest gram) under controlled lab conditions [47], which is considered one of the most reliable methods to measure dietary intake.

Diet quality was measured using questionnaires based on the FFQ in addition to National Healthy Eating Guidelines, Australian Guide to Healthy Eating, Amherst Health and Activity Survey of Child Habits, German Optimized Mixed Diet or Diet Quality Index-International (DQI-I) scores. In each case these consisted of components such as variety, adequacy, moderation, and balance in the diet. None of these measures were validated. Other non-validated measures included questions on fruit, vegetable, sweets, snacks (including salty) and carbonated drink consumption, in addition to regularity of breakfast consumptions and skipping meals (Eating Behaviour Questionnaire). Simple questions with questionable validity such as 'do you eat a healthy diet?' were also used.

Most of the studies used measures that relied on child/adolescent self-report. A few studies used a parent or caregiver to report their children's diet – this may affect the accuracy of the results due to social desirability factors or parents' lack of knowledge of what the child might be consuming away from home. Overall dietary intake was not measured in a coherent way and most tools used to measure nutritional intake or quality were not validated, particularly for the age range of the sample. Additionally, there was a lack of consistency between the studies with regards to the items of food used to define healthy or unhealthy diet and whether diet quality or dietary patterns should be used to best define an individual's dietary intake.

3.3.4 Measures of mood: A number of studies used measures of adolescent mental health that were well-established, validated and suitable for young people. The most common measures were the Child Behaviour Checklist (CBCL) [40, 41, 48], the Strengths, Difficulties Questionnaire (SDQ) [43, 45, 49-51], Short Mood & Feelings Questionnaire (SMFQ) [45, 52] and Depression self-rating Scale for Children (DSRS). The Center for Epidemiologic Studies Depression Scale for Children (CES-DC) was also used by one study [46]. These were typically completed by parents of younger children or by adolescents themselves. Some studies measured depression only and others used measures that were composed of components measuring both depression and anxiety ('internalising' problems).

A wide range of other measures were also used. Some were generic measure of adolescent well-being that included elements of depression e.g. Paediatric Quality of Life (PedsQL), some were completed by professionals on the basis of an unstructured consultation, e.g. International Classification of Disease (ICD-9/10), others were specific to depression but not designed for use by children and adolescents e.g. the Beck Depression Inventory (II), and the Depression Anxiety & Stress Scale (DASS-21). Additional *ad hoc* items such as "during the past 12 months, how often have you been so worried about something that you could not sleep at night?", "During the past 12 months, did you make a plan about how you would attempt suicide?" and questions on frequency of feeling depressed were also used but are of doubtful validity.

3.3.5 Study design: Most research studies used designs that were cross sectional. This is a relatively weak design because it is not able to determine the direction of the relationship between diet and mood. Longitudinal studies were uncommon. Socio-economic variables that are highly correlated with mood in children and young people and which are related to diet, such as SES, income and parents' educational level were not measured consistently and were not measured or not controlled by some [47,48,53-55]. The most common confounding variables that were controlled were the age and gender of participants. Some important variables such as medical conditions like hypothyroidism, diabetes, and food allergies, which may be correlated to mood or food choices, were not considered by any study.

Table 2: Key features determining the Quality ratings of the included studies

Citation	Participants		Diet		Mental Health		Respondent	Confounding	Quality
Country	Number	Age/year	Measure	Validated	Measure	Validated		Variables	Rating
Brooks et al., 2002 United states	2224	Mean: 16.2 (1.6)	Do you eat a healthy diet? Y/N.	No	Frequency of depression or 'stress' in the past 30 days.	No	Adolescents	Age, race and gender.	Fair
Fulkerson et al., 2004 United states	4734	Mean M: 14.9 (1.7) F:14.7 (1.7)	Meal, junk food, snacking frequency, YAQ.	Yes- YAQ	Kandel & Davies' 6-item scale.	No	Adolescents	Race and Grade level. Gender specific analysis.	Fair
Jacka et al., 2010 Australia	7114	Range: 10-14 Mean: 11.6 (0.81)	Unhealthy diet score based on dietary questionnaire.	No	SMFQ - 13 items.	Yes	Adolescents	Age, gender, SES, eating attitudes, PA, BMI and smoking.	Fair
Jacka et al., 2011 Australia	2915 After 2yrs: 2054	Range: 11-18	Healthy/ unhealthy diet based Dietary questionnaire.	Yes - in adults	PedsQL	Yes	Adolescents	Age, gender, SES, PA, dieting behaviours and BMI.	Good

Citation Country	Participants		Diet		Mental Health		Respondent	Confounding Variables	Quality Rating
	Number	Age/year	Measure	Validity	Measure	Validity			
Jacka et al., 2013 United Kingdom	2789	Range: 11-14	Unhealthy diet score: Frequency of fruit, vegetable, fast food snacks and breakfast.	No	SMFQ and SDQ	Yes	Adolescents	Gender, age, ethnicity, religion, SES, PA, BMI, dieting behaviour, alcohol, cigarette and drug use.	Good
Kohlboeck et al., 2012 Germany	3361	Range: 9.9-12.7 Mean: 11.15 (0.5)	Diet quality based on 82-item FFQ.	Yes FFQ	SDQ	Yes	Caretaker	Gender, SES, BMI, PA, television/PC use and total energy intake.	Fair
McMartin et al., 2012 Canada	3757	Range: 10-11	YAQ used to calculate DQI-I scores	Yes YAQ	ICD- 9 or 10	Yes	Child	Gender, energy intake, SES, weight, PA	Fair
McMartin et al., 2013 Canada	6528	Range: 10-11	YAQ used to calculate DQI-I scores	Yes YAQ	EuroQoL for youth.	Yes	Child	SES, height, weight, PA, gender and energy intake.	Fair

Citation Country	Participants		Diet		Mental Health		Respondent	Confounding Variables	Quality Rating
	Number	Age/year	Measure	Validity	Measure	Validity			
Mooreville et al., 2014 United States	Study 1: 228 Study 2: 204	Range: 8-17 Mean: 13 (2.8)	Energy (kcal) consumed after an overnight fast.	Yes	21- item BDI-II	Yes	Adolescents	Age, race , height, % fat mass and fat free mass.	Fair
Oddy et al., 2009 Australia	1324	Range: 13-15 Mean: 14 (0.2)	CSIRO FFQ used to identify healthy/ western dietary patterns	Semi validated in adults	CBCL (4-18yrs)	Yes	Caretaker	Total energy intake, PA, television/PC use, BMI, SES, and gender.	Fair
Oellinrath et al., 2013 Norway	1095	Range: 12-13	FFQ covering 40 food items	No	SDQ (parental version)	Yes	Caretaker	SES, PA, BMI and gender	Fair
Rao et al., 2015 Pakistan	4583	Range: 13-15	Frequency of carbonated drinks and fast food consumed.	No	Anxiety suicidal ideation measured using 1 item each.	No	Adolescents	Age, gender, BMI, SES, parental check, understanding, and close friends.	Poor

Citation Country	Participants		Diet		Mental Health		Respondent	Confounding Variables	Quality Rating
	Number	Age/year	Measure	Validity	Measure	Validity			
Renzaho et al., 2011 United states	3370	Range: 0-12	Frequency of Fruit & vegetable consumed.	No	SDQ	Yes	Caretaker	SES, age, gender, food security, social support.	Fair
Rubio-Lopez et al., 2016 Spain	710	Range: 6-9	3 day food diary.	Yes	CES-DC	No	Caretaker	Age, gender, BMI, SES and Nationality.	Fair
Robinson et al., 2011 Australia	1598	Mean: 14 (0.2)	CSIRO FFQ	Semi validated in adults	CBCL	Yes	Caretaker	Gender, family income, maternal employment.	Fair
Tajik et al., 2015 Malaysia	1568	Range: 13-14 Mean: 13 (0.8)	Eating Behaviour Questionnaire	No	DASS-21	Yes	Adolescents	No confounding variable identified.	Fair
Vollrath et al., 2011 Norway	40,266	1.5	Frequency of sweet food and drinks.	No	CBCL and EAS	Yes	Caretaker	Gender, weight, height and breastfeeding.	Fair

Citation Country	Participants		Diet		Mental Health		Respondent	Confounding Variables	Quality Rating
	Number	Age/year	Measure	Validated	Measure	Validated			
Weng et al., 2012 China	5003	Range: 11-16 Mean: 13.2 (0.99)	38-item comprehensive FFQ	No	DSRS Chinese version for children.	Yes	Adolescents	SES, age, gender, PA and BMI.	Fair
Wiles et al., 2009 United Kingdom	4000	Range: 3-4.5 Follow up age 7	FFQ: junk, health conscious & traditional	No	SDQ	Yes	Caretaker	Gender, SES, maternal depression & anxiety.	Good
Zahedi et al., 2014 Iran	13,486	Range: 6-18 Mean: 12.47 (3.36)	Frequency of junk food; sweets, beverages, fast foods and salty snacks.	No	Questions on depression insomnia confusion anxiety and aggression	No	Trained personal	Age, gender, family history of chronic diseases, mothers education, screen time, PA, SES and BMI.	Fair

Note: BDI-II Beck Depression Inventory; BMI= body mass index; CBCL= Child Behaviour Checklist; CES-DC= Center for Epidemiological Studies Depression Scale for Children; CSIRO= Commonwealth Scientific and Research Organisation; DASS= Depression, Anxiety and Stress Scale; DQI-I= Diet quality index-international; DSRS= Depression Self-rating Scale ; EAS= Emotionality Activity and Sociability Questionnaire; F= female; FFQ= Food frequency Questionnaire; ICD= International Classification of Diseases; M= male; PA= Physical Activity; PedsQL=Paediatric Quality of Life Inventory; SDQ= Strengths and Difficulties Questionnaire; SES= Socio-economic status; SMFQ= Short Mood and Feeling Questionnaire; YAQ=Youth and Adolescent Questionnaire.

3.3.6 Effect sizes: The association between diet and mental health was reported in a number of different ways. The most common method of evaluating the relationship between diet and mental health was to calculate the increased risk of depression given different types of diet. Other methods of analysis were univariate associations between the variables, multivariable linear regression and negative binomial regression with results reported as incidence rate ratio (IRR).

To allow the results of different studies to be compared on the same metric, we calculated effect sizes for all key variables where data were provided using the Practical Meta-Analysis Effect Size Calculator [56]. Two studies did not report data in a way that made this possible [40, 57].

3.3.7 Relationship between nutrition and mood: The main results of the 20 studies, including the effect sizes, are shown in Table 3. Due to the heterogeneity of the constructs, measurements, and definitions of both internalizing (depression and anxiety) symptoms and dietary intake e.g. quality, patterns, food groups and eating behaviours, the key results were grouped and described into the following broad categories:

3.3.7.1 Healthy Diet

3.3.7.1.1 Overall healthy diet: A 'healthy' diet was broadly defined as positive eating behaviours and consumption of fruits and vegetables, health promoting behaviours and avoiding 'unhealthy' food. However, there were inconsistencies regarding food items such as grains and legumes being part of a healthy diet. The relationship between healthy diet or healthy diet pattern and depression were investigated by eight studies [40,44-46,52-54,58]. Five studies reported a significant association between a healthy diet and lower depression with effect sizes ranging from small to medium (d = 0.5[52]). There was exception [40, 45, 46], where there was a weak eveidence for an association between healthy diet pattern and internalizing symptoms. One study [53] reported that the association between 'healthy' diet and mood was significant only for females (d=0.14). One research group explored the relationship between mental health and diet in a longitudinal design at two time points [44, 45]. Jacka et al. (2011) [44] found that that a healthy diet predicted depression two years later (d=0.43) but that depression at baseline did not predict healthy diet consumption (d=0.02) two years later. In contrast, Jacka et al., (2013) [45] found no association between a healthy diet and mental health 3 years later (d=0.11).

***3.3.7.1.2 Fruits & Vegetables*:** There were conflicting results regarding fruit and vegetable intakes, and their association with mood. The studies that explored this association [40, 41, 49, 50, 54, 57] all measured fruits and vegetables separately, except for two studies [49, 57], who grouped these

variables into a single category. Only one [50], investigated if mental health was associated with fruit and vegetable consumption. The majority of studies found no significant association between consumption of fruit and vegetable and mood. However, one study reported that compared to healthy individuals, individuals with emotional problems consumed significantly less fruit (in both males and females, average d=0.185) and vegetables (only in females, d=0.1) [50]. One other study reported that consumption of fruit and leafy green vegetables (only) was significantly associated with lower odds of internalising symptoms [40]. Other vegetables, such as cruciferous and yellow/red vegetables were not associated with internalising problems [40].

***3.3.7.1.3 Other food categories considered 'healthy'**:*

1. Cereal and grains: Two studies [41, 49] examined the effect of cereal consumption, whereas one [40] examined the effect of whole and refined 'grains' effect on mood. There was no evidence that cereal or grains were significantly associated with depression.
2. Dairy: All three studies [40, 41, 49] reported no significant association between dairy products and depression.
3. Fish: Four studies [40,41, 49, 57], explored fish intake and its association with depression. However, only one [57] reported higher fish consumption to be significantly associated with decreased odds of developing mental health difficulties.

3.3.7.2 Unhealthy Diet

***3.3.7.2.1 Overall unhealthy diet**:* Six studies investigated the relationship between unhealthy diet and mental health [40, 42, 44, 52, 54]. An 'unhealthy' diet was broadly defined as one comprised of fast foods or take away, foods containing high fat and sugar levels, confectionery, sweetened beverages, snacking, Western dietary patterns, and unhealthy food preferences. Typically, 'unhealthy' diets were reflected in a continuous score with higher levels indicating an unhealthier diet. Each of the studies reported a significant cross-sectional association between unhealthy diets and depression, with small to moderate effect sizes (d = 0.1 to 0.39). Jacka and colleagues explored the link between mental health and an unhealthy diet in a longitudinal design. They found that unhealthy (d=0.26) diet at baseline significantly predicted the occurrence of depression two years later [45] but did not predict depression at 3 years (d=0.097) [47]. They also reported no association between depression at baseline and unhealthy diet consumption over time (d=0.06) [44].

3.3.7.2.2 Fast Food/Take away/ eating away from home/ junk food: Seven studies investigated junk food or fast food consumption and mental health in adolescents [40, 41, 43,46, 54,55, 59]. Food items within this category consisted of Western food items or processed foods such as hamburger, pizza, meat pie, savoury pastry, meat pies, fried food, hot chips, coated poultry, and soft drinks. The food items included were more or less similar for different countries and cultures. Four studies [40, 41, 55, 59] reported an association between high take-away/fast food consumption and increased odds of mental health problems. Overall, the effect sizes of these studies were small. With the exception of one study [41] which included confectionary and snacking as a part of junk / fast food consumption and therefor reported a larger effect size of junk food on mental health (Table 3). One study [43] used a longitudinal design, with dietary consumption measured by parental report at 4.5 years and parent reported mental health problems at the age of 7. Consumption of junk food at 4.5 years did not predict emotional problems at 7 years.

3.3.7.2.3 Snacking*:* Snacking was defined as the consumption of the following food items between meals: preserved fruits, confectionery, crisps, ready to eat savouries, salty snacks, and carbonated beverages etc. Five studies examined the relationship between snacking and depression [40, 46, 49, 58, 59]. Only two studies [58,59] reported a significant association between snacking and depression, with small effect sizes (d = 0.05 and 0.12).

3.3.7.2.4 Confectionery/sweets: This category is divided into sweet foods, such as confectionery, cakes, biscuits, and sweet drinks, such as soft drinks and sweet beverages. Four studies examined the relationship between confectionery or sweet foods and depression in a cross-sectional design, [40, 43, 47, 49] of which three [43, 47, 49] found a significant cross-sectional association. In a longitudinal study there was no significant association between sugar consumption and mental health after 3.5 years [43]. One study [40] found no association between consumption of baked goods and depression but reported a significant association between confectionery consumption and increased odds of depression, the effect size was however very small (d=0.04). Three studies [40, 54, 59], also investigated the effects of sweet drinks on mood. Daily consumption of sweet drinks was significantly associated with increased depressive symptoms in all 3 studies the effect sizes of these studies were small (d=0.09 – 0.25) and in one study [54] the effect was significant only in males (d=0.25). One study [48] explored the association between mental health on consumptions of sweet foods and drinks and reported that individuals with poorer mental health were more likely to consume sweet food and drink.

3.3.7.2.5 Meat: The association between meat consumption and mental health was investigated in four studies [40, 41, 49, 58]. Three [40, 41, 49] investigated the effects of red meat and meat products, one [58] explored the effect of 'animal' dietary pattern, consisting of processed meat and other meats on mental health. Only one of these four studies [40] reported that high meat consumption was significantly associated with poorer mental health.

3.3.7.2.6 Other food categories considered unhealthy:

1. Fats: Three studies ivestigated fat intake, one explored intake of fats and oils [49] and two [54,57,] reported total percentage fat intake. These 3 studies did not find a signification association between fat consumption and depression.
2. Caffeine: Only one study examined the relationship between caffeine and mood and found that caffeine was significantly associated with depression (average d=0.37) [54].

3.3.7.3 Overall Diet Quality: In addition to 'healthy' and 'unhealthy' diet being investigated separately, the association between overall diet quality and depression has also been explored [49, 57, 60]. Two studies [49, 60], reported an association between higher diet quality scores and depression with a small effect size (d = 0.025 and 0.03, respectively). One study reported no significant association between depression and overall diet quality; however, they did report that greater variety and adequacy of the diet was significantly associated with a lower level of emotional problems (unable to calculate effect size) [57].

3.3.7.4 Eating Behaviours: The relationship between depression and 'eating behaviours' such as having breakfast, lunch, dinner and skipping meals was explored in two studies [54, 55]. Both reported significant associations between having breakfast and lower depressive symptoms (d= 0.31 [54], d= 0.03[55]). However, there were conflicting results regarding lunch and dinner consumption. One study showed an association between higher depression symptoms and individuals who skipped dinner (average d= 0.28) or lunch (average d=0.34) [54]. The second study found no significant association between depressive symptoms and having lunch (d=0.03) or dinner (d=0.048) [55].

3.3.7.5 Overall Dietary Intake: A recent study investigated the association between self-reported depressive symptoms and 29 different nutrients (including macro, micronutrients, and minerals) [46]. Intake of protein, carbohydrates, pantothenic acid, biotin, vitamin B12, vitamin E, zinc, manganese, cobalt, aluminium, and bromine was significantly lower in children with depressive symptoms. Whilst consumption of thiamin and vitamin K was high in children with depressive

symptoms when compared to non-symptomatic peers. However, the effect sizes for the significant results were small ranging from *d*=0.18-0.21, with the exception of biotin (d=0.99). The list of all the nutrients and their effect sizes is reported in Table 3.

Additionally, two further studies have investigated a few specific nutrients in addition to exploring overall 'diet'. Fulkerson and colleagues investigated, calcium, iron, sucrose, vitamin D, folate, vitamin B6 and B12 [54], whilst McMartin investigated the intake of omega-3 fatty acid and the ratio between omega-3 and omega-6 [57]. Neither study found an association between consumption of any of these nutrients and mental health problems.

3.3.7.6 Diet and Anxiety: Three studies [42, 585, 59] explored the association between diet and anxiety alone, with one [58] also exploring the relation between comorbid depression and anxiety. There was a significant association between anxiety and three or more unhealthy behaviours, such as consumption of fast food and sweet beverages [42]. Another study [59] reported that consumption of sweets, sweet beverages, fast food, and salty snacks was associated with increased odds of anxiety. However, the effect sizes of both of these studies was small (d=0.21 and d=0.21 Table 2). Higher consumption of 'animal' food types and 'snacking' dietary patterns was associated with anxiety and comorbid depression [58]. A traditional dietary pattern, consisting typically healthy foods such as fruits, vegetables, oatmeal and wholegrain, was negatively associated with coexisting depression and anxiety (d=0.04) but not with anxiety alone.

Table 3: Key results.

Citation	Study Design	Key results: Description and Effect sizes
Brooks et al., 2002	Cross sectional	Healthy diet negatively correlated with depression only in females: ^{males}d=0.016 ^{females}d=0.028*
Fulkerson et al., 2004	Cross sectional	Health promoting attitude negatively correlated with depression ^{males} d=0.51*, ^{females}d=0.28*. Health compromising attitude positively correlated with depression ^{males} d=0.34*, ^{females}d=0.19*. Breakfast consumption negatively correlated with depression ^{males} d=0.32* ^{females}d=0.30*. Lunch, ^{males}d=0.29*,^{females}d=0.39*, and dinner, ^{males} d=0.25*,^{females} d=0.30*, negatively correlated with depression. Daily consumption of soft drinks positively correlated with depression in males only ^{males}d=0.25*, ^{females}d=0.09. Caffeine intake positively associated with depression ^{males}d=0.41*, ^{females} d=0.33*. Not significantly associated with depression: Snacking in between meals ^{males}d=0.085, ^{females}d=0.084, fast food consumption ^{males}d=0.12 , ^{females}d=0.10, daily vegetable intake ^{males}d=0.06, ^{females}d=0.11, daily fruit intake ^{males}d=0, ^{females}d=0.1, calcium ^{males}d=0.031, ^{females}d=0.04, iron ^{males}d= 0.05, ^{females} d=0.011, ^{males}d=0.15, ^{females}d=0.044, Vitamin D ^{males}d=0.051, ^{females}d=0.068, Folate ^{males}d=0.049, ^{females}d=0.069, Vitamin B6 ^{males}d= 0 ^{females}d=0 and Vitamin B12 ^{males}d= 0.06, ^{females}d=0.034.
Jacka et al., 2010	Cross sectional	Healthy diet negatively correlated with depression d=0.55* Unhealthy diet positively associated with depression: d=0.39*
Jacka et al., 2011	Cross sectional	Healthy diet negatively correlated with depression d=0.286* Unhealthy diet negatively associated with depression d=0.181*
	Longitudinal	Diet predicted mental health at 2 year follow up: Healthy diet score d=0.43*, Unhealthy diet scores d=0.26* Mental health did not predict diet at 2 year follow up: Healthy diet score d=0.02, Unhealthy diet scores d=0.06
	Cross sectional	Healthy diet was not correlated with psychological distress SDQ (d=0*) and SMFQ (d=0.001*)

Citation	Study Design	Key results: Description and Effect sizes
Jacka et al., 2013		Unhealthy diet positively correlated with psychological distress SDQ (0.178*) and SMFQ (d=0.099*)
	Longitudinal	Unhealthy (d=0.097) and healthy diet scores (d=0.111) did not significantly predict mental health at 3 years.
Kohlboeck et al., 2012	Cross sectional	Higher diet quality significantly negatively associated with emotional problems (d=0.03*) Confectionary significantly positively associated with emotional problems (d=0.04*) Not associated with emotional problems: Bakery wares (d=0.01), Fats and oils (d=0.05), Dairy products (d=0.025), Meat and meat products(d=0.03), Cereals(d=0.013), Eggs (d= 0.012), Fruit and vegetables (d=0.012), Fish (d=0.002), Ready to eat savouries (d=0.009) and Beverages (d=0.005).
McMartin et al.,2012	Cross sectional	Not enough information to calculate effect size. Variety and increased adequacy in diet significantly associated with internalising disorder (IRR=0.45* CI=0.25, 0.82 and IRR=0.64* CI=0.34, 1.2 respectively). Not associated with emotional problems: Overall diet quality not significantly associated with internalising disorder (IRR=1.09 CI=0.73, 1.63) Moderation in diet not associated with internalising disorder (IRR=1.07 CI=0.66, 1.73). Balance in diet not associated with internalising disorder (IRR=1.06 CI=0.66, 1.73). Fruit and vegetables (IRR=1.25 CI=0.80, 1.99), Folate (IRR=1.21 CI=0.64, 2.32), Vitamin B6(IRR=1.05 CI=0.56, 1.99), Vitamin B12 (IRR=0.77CI=0.5, 1.17), Fish intake (IRR=0.59 CI=0.41, 1.55), n-3 fatty acid (IRR=0.97 CI=0.61, 1.55), n3:n6 ratio (IRR=0.9 CI=0.67, 1.21), percentage energy from fat(IRR=0.82 CI=0.55, 1.22).

Citation	Study Design	Key results: Description and Effect sizes
McMartin et al.,2013	Cross sectional	Diet quality negatively associated with worrying, sad or unhappy feelings (d=0.025*). Higher variety in diet negatively associated with worrying, sad or unhappy feelings (d=0.012*). Increased adequacy in diet negatively associated with worrying, sad or unhappy feelings (d=0.028*). Balance in diet was significantly associated with worrying, sad or unhappy feelings (d=0.012*). Moderation in diet not associated with worrying, sad or unhappy feelings (d=0.01).
Mooreville et al., 2014	Cross sectional	Study 1: Depressive symptoms not associated with consumption of sweet snacks (d=0.26). Study2: Depressive symptoms associated with consumption of sweets snack (d=0.52*)
Oddy et al., 2009	Cross sectional	Not enough information to calculate effect size. Leafy green vegetables b=-1.98*(CL= -3.80 to -0.16] and fruit: b=-2.16*(CI= -3.92 to -0.41)] associated with lower internalising score. Western dietary pattern overall significantly associated with 'internalising' symptoms b=1.25*(CI=0.15-2.35). Takeaway [Q4: b=1.89*(CI0.07-3.71)]; Confectionary [Q4: b=2.63*(CI0.87-4.39)] and Red meat [Q4: b=1.98*(CI=0.20-3.76)] significantly associated with higher internalising scores. Not associated with emotional problems: Healthy diet pattern overall not significantly associated with Internalising symptoms b=1.25 (CI= -0.54,0.88). Tomato, yellow/red b= −0.51(−2.24, 1.23), cruciferous, other vegetables, legumes, whole grains and fish steamed grilled or tinned not associated with internalising symptoms. Refined grains, processed meat, potato fried, crisps, soft drinks, cakes/biscuits, sauces/dressings and full fat dairy products not associated with internalising symptoms.
Oellinrath et al., 2013	Cross sectional	Not associated with problems: Junk/convenient (d=0.097), Varied Norwegian (d=0.053) and Snacking (d= 0.025).

Citation	Study Design	Key results: Description and Effect sizes
Rao et al., 2015	Cross sectional	Positive correlation between anxiety and three [d= 0.14*], or four or more [d= 0.21*] unhealthy behaviours. Positive correlation between suicidal ideation and two (d=0.10*), three (d=0.27*) or four or more (d=0.36*) unhealthy behaviour.
Renzaho et al., 2011	Cross sectional	Fruit consumption negatively associated with emotional problems in males (d=0.16*) and females (d = 0.21*). Vegetable consumption negatively associated with emotional problems in females (d= 0.1*) not, males (d=0.03).
Robinson et al., 2011	Cross sectional	Takeaway and snacks positively associated with higher internalising symptoms (d = 1.0*). Cereals (d=0.27), fruits (d= 0.27), dairy (d = 0.33), meat/meat alternatives (d=0.03) and vegetables (d=0.42) not significantly associated with internalising symptoms.
Rubio-Lopez et al., 2016	Cross sectional	Nutrients lower in depressed group: proteins (d=0.215*), carbohydrates (d=0.185*), pantothenic acid (d=0.188*), biotin (d=0.994*), vitamin B12 (d=0.222*), vitamin E (d=0.229*), zinc (d=0.280*), manganese (d=0.209*), cobalt (d=0.249*), aluminium (d=0.216*) and bromine (d=0.182*). Nutrients higher in depressed group: thiamine (d=0.185*) and vitamin K (d=0.282*). Not associated with depression: Lipids (d=0.0005), Fibre (d=0.149), Riboflavin (d=0.08), Niacin (d=0.105), Vitamin B6 (d=0.165), Folic acid (d=0.128), Vitamin C (d=0.181), Vitamin A (d=0.181), Vitamin D (d=0.089), Calcium (d=0.026), Phosphorus (d=0.013), Iron (d=0.143), Iodine (d=0.163), Fluoride (d=0.106) and Selenium (d=0.077).
Tajik et al., 2015	Cross sectional	Eating out of home 4-7 times a week positively associated with higher levels of depressive symptoms (d=0.08*) Breakfast more than 4 days a week associated with lower depressive symptoms (d=0.03*) Lunch (d=0.03) and dinner (d=0.048) not significantly associated with depressive symptoms.
Vollrath et al., 2011	Cross sectional	Internalising problems are positively associated with being fed more high calorie drinks at night (d=0.26*), sweet food (d=0.09*) and sweet drinks(d=0.14*).

Citation	Study Design	Key results: Description and Effect sizes
Weng et al., 2012	Cross sectional	Snacking pattern positively associated with depression (d=0.12*) and anxiety (d=0.15*) Traditional dietary pattern negatively correlated with depression (d=0.23*) but not with anxiety (d=0.04). Animal dietary pattern not associated with depression (d=0.05) but associated with anxiety (d=0.15).
Wiles et al., 2009	Longitudinal	Junk food (d=0.002) and sugar intake (d=0.0) at age of 4.5 not associated with emotional problems at age 7.
Zahedi et al., 2014	Cross sectional	Consumption of sweets (weekly d=0.03*, daily d=0.01*) sweetened beverages (weekly d=0.01*, daily d=0.08*) fast food (weekly d=0.02*, daily d=0.095*) and salty snacks (weekly d=0.01*, daily d=0.05*) positively associated with depression. Consumption of sweets (weekly d=0.05*, daily d=0.03*) sweetened beverages (weekly d=0.03*, daily d=0.09*) fast food (weekly d=0.02*, daily d=0.08*) and salty snacks (weekly d=0.005*, daily d=0.05*) positively associated with anxiety

Note: C = Category, CI = Confidence Interval, IRR = Interval Risk Ratio, OR = Odds Ratio, PedsQL = Paediatric Quality of Life Inventory, Q = Quartile, SDQ = Strengths and Difficulty Questionnaire, SMFQ = Short Mood and Feelings Questionnaire, * = Significant Result

3.4. Discussion

This systematic literature review identified and evaluated research examining the relationship between diet and mental health in children and adolescents. The current first line treatment for depression are antidepressants that act through dopaminergic, serotonergic, and monoaminergic mechanisms. These however fail to decrease the burden of depression due to people's lack of response to these medications, especially the younger population [61]. This suggests that there may be an alternative mechanism through which depression can be targeted. Any possible method of preventing the development of depression symptoms or reducing existing symptoms has great potential as a public health intervention. In addition to nutrition, other methods that are being investigated includes psychoactive compounds, such as agomelatin, that synchronizes circadian rhythms, targeting inflammation and gut microbes, all of which are beyond the scope of this review [62-65].

Despite the importance of the topic, we found relatively few studies that examined diet and mental health in adolescents, especially when compared with the large number of studies with adult participants.

Our review highlights a number of important issues, both methodological and substantive. From a methodological perspective there are significant problems in the design and conduct of epidemiological studies. Although only 20 studies were identified, a range of different ways of defining and conceptualising diet quality were used that could not be easily compared or integrated. Even well-established measures of diet quality relied on retrospective self-report of food consumption, which is of dubious reliability and validity. The more intrusive but reliable use of daily food diaries was rarely reported. The measurement of depression and associated mental health difficulties was somewhat more satisfactory in that some well standardised and validated measures with good psychometric qualities were used.

Of more concern, however, is the related problem of study design, all of the studies identified in this review were correlational. Only 3 included a longitudinal element, and thus most could not help determining the direction of the causal relationship between diet and mood [43-45]. Intervention studies, using an overall diet strategy are the only robust way to establish causality; if these are impractical or impossible to conduct then it is essential to conduct careful longitudinal studies with adequate methods of measuring key constructs of diet and mood. Further adding to the difficulty in understanding any causal relationship between diet and mental health is that both diet and mood are influenced by many other factors including socio-economic status, culture, and age [33,66-69]. Few studies attempted to control the impact of important confounds and thus any observed relationships between diet and mental health

must be interpreted cautiously. It is entirely plausible that low mood and poor diet are both caused by the same third variable, low socio- economic status or social exclusion, both of which would act to restrict access to a varied healthy diet and to increase adverse life and other environmental causes of poor mental health.

These methodological problems made it difficult to integrate the studies and to make inferences regarding the association between mood and dietary pattern. Most studies included multiple measures of 'diet' quality or content and included multiple significant testing, thus increasing the likelihood of Type I and II errors. To impose some consistency on the results of multiple statistical tests using different measures of diet and mental health, we calculated the effect sizes for each study. Given the caveats outlined above relating to methodological and conceptual problems, there was a general tendency to report small associations between diet and mental health, with 'unhealthy' diet associated with increased odds of mental health difficulties, and 'healthy' diet having the opposite effect. Similar conclusions were drawn in the studies investigating whether healthy and unhealthy diet is associated with depression in adults [21-28]. These results contradicted the finding from a recent review where consistent association between unhealthy dietary pattern and worsening of mental health was observed, however the same was not observed for healthy diet and better mental health in young people [34].

No inferences could be made about the association between fast food, vegetables and fruits and mental health. Therefore, because causality cannot be determined, it is important to note that there is a plausible alternative causal pathway whereby low mood leads to increased consumption of unhealthy 'junk' food e.g. chocolate and decreased consumption of 'healthy' foods. These conflicting and heterogeneous findings were similar to those found in adult studies. A recent review of adult literature also identified similar problems regarding the method quality and the inconsistencies between the constructs [70].

Given the inherent limitations of cross-sectional research designs and the demands of large community intervention studies, another tactic may be to focus on observational and intervention studies with 'at risk' or clinical populations. This could involve comparisons of nutritional intake between healthy adolescents and those with anxiety and depressive disorders. However, to make any confident causal statement about the effects of diet on mental health, intervention studies are required, and these can be best informed by theory about mechanisms and better designed correlational studies.

Overcoming the methodological problems discussed in this review will require greater collaboration and communication between researchers. This will help to establish clearer and

more consistent definitions and constructs, and more shared use of reliable and valid instruments that can be used consistently across cultures, communities, and cohorts.

3.5 Conclusion

Research regarding dietary pattern, diet quality and its association with mental health in children and adolescents, is at an early stage. This review highlighted some conceptual and methodological problems that, if not addressed, will impede future research and public health interventions. It is therefore essential to make sure that further methodological problems are minimized to at least establish the strength of any association between diet and mental health.

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Chapter 4: Study 2: Differences in Nutritional Intake Between Healthy and Low Mood Adolescents: A Pilot Study

Abstract

Associations between diet quality and depression have been explored in the past decade. In adults, there is a significant inverse correlation between a good quality diet and depressive symptoms. However, there are a lack of studies, particularly in adolescents from the United Kingdom (UK). This exploratory study aimed to investigate the association between diet quality and symptoms of depression in adolescents from the UK. Seventy-seven 11-17-year-old participants were recruited (47 female). Depressive symptoms were assessed using the Mood and Feelings Questionnaire. Twenty-three participants had elevated symptoms of depression and fifty-four had depression within the non-clinical range. Nutritional profile was measured using European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaire. Consumption of 46 nutrients including macro and micronutrients in both groups was calculated and used to calculate diet quality. Diet Quality Index-International was used to then calculate diet quality scores. Variety, adequacy, moderation, and balance in the diet was assessed to calculate the diet quality score. A Multiple linear regression model showed no significant association between variety, adequacy, moderation, and balance in diet with depressive symptoms in adolescence, explaining only 1% of the variance. Adequacy was the strongest predictor in the model, with variety in diet being the weakest. Overall, the mean DQI-I score of this sample indicated average quality diet with best achieving score being adequacy, followed by variety and moderation. Overall balance in diet was the weakest scoring component. Participants consumed over 50% of the recommended amounts of nutrients making up the adequacy components except for fruits. 83% of the adolescents failed to consume the daily recommended amounts. Additionally, 70% of the participants consumed empty calories above the recommended levels, and none of the participants achieved the suggested carbohydrate to protein to fat ratio in diet. Future studies should include a larger sample size and include a longitudinal follow-up to explore if healthy participants who do not meet the recommended amount of daily nutritional intake are at increased risk of developing symptoms of depression. Studies should also explore the diet quality of adolescents who are at risk of, and suffer from depression, to get a holistic picture of the areas of a diet that are associated with depression.

4.1 Introduction

Major Depressive Disorder (MDD) is a chronic and disabling disorder characterised by low mood/irritability and/or loss of pleasure in combination with sleep, appetite, cognitive and energy changes. It is also often accompanied by feelings of guilt, worthlessness, and suicidal thoughts. MDD is projected to produce the second greatest impact on disability-adjusted life years and a significant socioeconomic burden by the year 2020 [(1)]. It has an early onset often beginning during childhood or young adulthood [(2)], adolescence being one of the greatest periods for development of depression [(3)]. Depression in pre-pubertal children is rare, with prevalence increasing markedly between the ages 13 and 18, effecting more women compared to men [(4)]. Approximately 2.6% of young people are reported to experience an episode of depression at any one time [(5)]. Adolescent depression is associated with increased risk of depression and suicidal ideation in later life [(6,7)]. Despite the advances in treatment of mental disorders, depression is now among the top ten leading causes of disability globally. It is therefore crucial to investigate alternative preventative and treatment strategies especially in young people, as not only it is an important developmental period but also evidence-based treatments in this group is limited.

The role of nutrition in the development of depression has recently been of interest. It has been suggested that dietary strategies could play an important role in treatment and prevention of depression. Evidence suggests that certain nutrients may be important in preventing the development of major depressive disorder. For example, depression is common in individuals with low concentrations of fatty acids in their red blood cells, reflecting their level of composition in the brain [(8-10)]. Some studies have shown an inverse relationship between omega-3 fatty acid levels, found naturally in oily fish, walnuts, flaxseed oil and soybeans, and severity of depression [(9,11)] and self-harm [(12,13)]. It is hypothesised that essential fatty acids, such as omega 3, enhance membrane fluidity in the brain, resulting in increased transport of serotonin, a neurotransmitter that influences mood [(14-16)]. Depression in adults has also been associated with low levels of some vitamins and minerals. Levels of folic acid (vitamin B9), which is present naturally in a variety of foods, e.g. leafy green vegetables, are inversely correlated with severity of depression [(17,18)] and low levels of folic acid predicted a poor response to antidepressants [(19)]. Vitamin B12, present naturally in fortified foods such as cereals and soy products, also in fish and beef liver, was lower in depressed adults than in healthy adults [(20,21)]. Minerals such as selenium and zinc have also been reported to be significantly lower in depressed individuals compared to healthy adults [(22-24)]. The role of diet and mental health has not been widely investigated in children and adolescents. Overall dietary habits (e.g. consumption of ‘junk’ food vs. healthy foods) have been investigated in

community based epidemiological studies, in young people at familial risk of depression and in non-treatment seeking young adults. Unhealthy eating, assessed by the frequency of certain food groups such as sweets, cakes, soft drinks, and low levels of fruits and vegetables, was associated with mental disorder, psychiatric distress in children, and problematic behaviours in adolescents [25-28].

These studies suggest that there is a general relationship between mood and nutrition in adolescents and children. A recent literature review concluded that attempting to create a physiological change via the prescription of a single nutrient is ambitious. As the nature of overall nutritional intake and internal biochemical mechanisms of metabolism is complex in nature, studies looking into detailed nutritional profiles are of great importance. It was reported that, although there were studies looking into overall dietary patterns, there were inconsistencies in the way terms such as 'healthy' and 'unhealthy' diets ware defined [29]. A few studies have investigated traditional diets, such as, Mediterranean, Norwegian, Japanese, and Western diets, and their effect on mental health [30]. This again poses the limitation of how these diets are defined and are specific to certain cultures. Food intake patterns are likely to be more heterogeneous globally than nutrient intake patterns. Hence, measuring diet quality using diet quality index, based on the food group composition and intake of nutrients associated with chronic disease or diet variety, has attracted growing interest [31]. There are mathematical algorithms aimed at quantifying the extent to which nutrient intake complies with the reference intake values recommended in the national dietary guideline. Though the concept of diet quality is heterogeneous and multidisciplinary, it measures overall nutritional intake from a diverse perspective and in a comprehensive manner. Taking into account that the type of food items consumed differs for every country and culture, and only a few studies have examined in detail the diet of adolescents in the UK and associations with mood, the aim of this exploratory pilot study is therefore, to investigate the diet of adolescents in the UK and determine the association between diet quality and depressive symptoms using a diet quality index.

4.2. Methods

4.2.1 Ethics: This research was reviewed and given a favourable ethical opinion for conduct by the University of Reading Research Ethics Committee.

4.2.2 Participants and Procedure: Participants were young people aged 11-17 years of varying ethnicity and residing in the UK. They were recruited via schools in Reading Berkshire, UK and advertisements at university open days. Due to the lack of existing studies

to help calculate the number of participants required, especially those with a case-control study design, a g* power calculation was not appropriate. A total of 146 participants were recruited, of whom 69 dropped out (i.e. did not return their questionnaires) and were excluded from the study. This resulted in 77 participants who were then included in the analysis. All parents or legal guardians provided informed written consent for young people under the age of 16. Participants under the age of 16 provided written assent and those over 16 gave written consent.

4.2.3 Measures: Depression symptoms were assessed using the Mood and Feelings Questionnaire (MFQ) [(32)] (Appendix 2). The MFQ is a standardized and well-validated 33-item self-report measure of the severity of depression symptoms in adolescents. Each item relates to a symptom or experience associated with depression. Participants are asked to rate each item in relation to their symptoms in the past 2 weeks on a 3-point Likert scale (not true -0, sometimes -1, true -2). Total MFQ scores range from 0 to 66 where higher scores indicate greater risk of depression. A score of 27 or above is the clinical cut off to identify young people who are considered to be at risk of depression and those who score 14 or below on the MFQ are considered healthy. These were the guidelines used to determine whether a participant should be in the healthy or low mood groups, with young people scoring between 14-27 being excluded from the study.

Dietary intake was measured using the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Food Frequency Questionnaire [(33)] (Appendix 3), which is a semi-quantitative questionnaire that was used to record the average intake of food during the previous month. This is a validated self-report measure of dietary intake in children, adults and the elderly. The questionnaire contains a list of 130 food items. For each item participants are asked to indicate their usual rate of consumption from nine frequency categories ranging from “never or less than once per month”, “1-3 times per month”, “once a week”, “2-4 times per week”, “5-6 times per week”, “once a day”, “2-4 times per day”, “4-5 times per day” and “6+ times per day”. The servings were specified in terms of units, common portions, and household measures such as one apple or one slice of bread, half cup of rice or a spoon of French dressing. Additionally, minor modifications were made to make it easier for adolescents to complete i.e. portion sizes are specified on each food items and portions sizes were illustrated with photographs. The FFQ is linked to a web based nutritional calculator called FETA (FFQ- EPIC Tool for Analysis) [(33)], which provides extensive data about the nutrient and food group intake values consumed by participants. In total, 46 nutrients intake were calculated which include several carbohydrates (total, fructose, glucose, galactose, lactose, maltose, sucrose, starch, total sugars and Non Starch Polysaccharides-NSP), fats

(Monounsaturated fatty acids – MUFA, Polyunsaturated fatty acid – PUFA, saturated fatty acids -SFA and cholesterol), proteins, vitamins (A, B1, B2, B6, B12, C, D, E and total folate), precursors of vitamin A (Total Alpha and Beta Carotene), minerals/elements (Calcium, Chloride, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Niacin, Phosphorus, Selenium, Nitrogen and Zinc), in addition to total energy intake. The food groups included alcoholic beverages, non-alcoholic beverages, cereals & cereal products, eggs & egg dishes, fats & oils, fruits, vegetables, meat & meat products, milk & milk products, nuts & seeds, potatoes, soups & sauce, and sugars & snacks.

Diet quality index was measured using Diet Quality Index-International (DQI-I) [(34)]. The DQI-I includes four major aspects of a high-quality diet which includes variety, adequacy, moderation, and overall balance. The components that make up these categories of diet quality are shown in Table 1. Scores and scoring criteria for each component are also summarized in Table 1. Scores from all components are summed and result in a score ranging from 0 to 100, lower scores indicating poorer diet quality.

Table 1. Components of Diet Quality Index-International (DQI-I), their scoring criteria and range.

Component	Score ranges	Points	Scoring criteria
1.VARITEY	0-20		
Overall food group variety [a]	0-15	15	≥1 serving from each food group/d
		12	Any 1 food group missing/d
		9	Any 2 food group missing/d
		6	Any 3 food group missing/d
		3	≥4 food group missing/d
		0	None from any food group/d
Within-group variety from protein source [b]	0-5	5	≥3 different sources
		3	2 different sources
		1	1 source
		0	None
2.ADEQUACY	0-40		
Vegetable	0-5	5	≥3-5 servings
		3	1-2 servings
		0	0 serving
Fruit group	0-5	5	≥2-4 servings
		3	1 serving
		0	0 serving

Component	Score ranges	Points	Scoring criteria
		5	≥6-11 servings
Grain group	0-5	3	1 serving
		0	0 serving
		5	≥20-30g/d
Fibre	0-5	3	<20-30g/d
		0	0 g/d
		5	≥10% of total energy/d
Protein	0-5	3	<10% of total energy/d
		0	0% of the total energy/d
		5	≥100% of RDA
Iron	0-5	3	<100% of RDA
		0	0% of the RDA
		5	≥100% of RDA
Calcium	0-5	3	<100% of RDA
		0	0% of the RDA
		5	≥100% of RDA
Vitamin C	0-5	3	<100% of RDA
		0	0% of the RDA
3. MODERATION	0-30		
		6	≤20% of total energy/d
Total Fat	0-6	3	>20-30% of total energy/d
		0	>30% of the total energy/d
		6	≤7% of total energy/d
Saturated Fat	0-6	3	>7-10% of total energy/d
		0	>10% of the total energy/d
		6	≤300mg/d
Cholesterol	0-6	3	>300-400mg/d
		0	> 400mg/d
		6	≤ 2400mg/d
Sodium	0-6	3	>2400mg/d
		0	> 3400mg/d
		6	≤3% of total energy/d
Empty Calorie Food	0-6	3	>3-10% of total energy/d
		0	>10% of the total energy/d
4. OVERALL BALANCE	0-10		
Macronutrient ratio	0-6	6	55–65:10–15:15–25

Component	Score ranges	Points	Scoring criteria
(carbohydrate:		4	52–68:9–16:13–27
protein: fat)		2	50–70:8–17:12–30
		0	Otherwise
Fatty acid ratio (PUFA, MUFA, SFA)	0-4	4	P/S= 1–1·5; M/S=1–1·5
		2	P/S=0·8–1·7; M/S=0·8–1·7
		0	Otherwise

M/S, ratio of MUFA to SFA intakes; P/S, ratio of PUFA to SFA intakes; SFA, saturated fatty acids.
[a] Food groups include: 1. meat/poultry/fish/eggs, 2. dairly/beans, 3. Grains, 4. vegetables, 5. fruits.
[b] Protein sources include meat, poultry, fish, dairy, beans, and eggs

4.2.4 Statistical Analysis: All statistical analysis was conducted using IMB SPSS version 22. The relationship between age and depressive symptoms was assessed using Pearson's correlation. Multiple linear regression was performed with MFQ scores as the dependent variable and DQI scores, variety score, adequacy score, moderation score and balance scores as the predictor variables. One sample t-test was used to compare the amount of fruit and vegetable intake of participants with the daily recommended amount, as suggested by the Public Health England.

4.3 Results

Seventy-seven participants were recruited (47 females) aged 11-17-year olds (M=14.91 years, SD=1.88). Fifty-four participants scored below the clinical cut off on the MFQ and twenty-three participants reported elevated symptoms of depression. The overall range of the MFQ scores were from 1 to 55, M = 15.37(SD = 12.66). There was no significant correlation between age and MFQ scores (r=0.16 N=76 p=0.16).

Table 2. Showing the number of male and females with and without symptoms of depression.

	Healthy (MFQ score < 14)	**Elevated Symptoms (MFQscore >27)**	**Total**
Male	21	7	28
Female	33	14	47
Total	54	21	75

The mean total DQI-I scores were 59.9% of the possible score of 100%. The mean scores of overall diet quality and its components are summarised in Table 2. The best achieved score was adequacy (71.25%), followed by variety (64%) and moderation scores (61.6%). Overall,

balance was the weakest area of the diet (1%). From the variety category only 12% consumed at least one serving from each food group, 34% of adolescents missed only one food group and 38% missed two food groups. Additionally, only 25% had three or more different sources of protein per day. According to the adequacy category participants consumed over 50% of the recommended amounts of vegetable, grains, fibre, protein, iron, calcium, and vitamin C. However, the amount of fruits consumed was lower than 50% of the recommended amount (36%). 83% and 52% of adolescents failed to meet the recommended levels of fruit and vegetable intakes, respectively. One sample t-test was used to compare the consumption of grains, fibre, protein, iron, calcium, vitamin C, fruits, and vegetables to the recommended daily intake in young people. The mean scores are reported in Table 3. Overall, the participants significantly consumed lower amount of these nutrients than the daily recommended amount, vitamin C, $t(76)=3.17$ $p=0.002$, calcium, $t(76)=17.7$ $p<0.005$, iron, $t(76)=19.6$ $p<0.005$, protein, $t(75)=7.8$ $p<0.005$, fibre, $t(76)=21.35$ $p<0.005$, grains, $t(76)=6.77$ $p<0.005$, fruits, $t(76)=14.16$ $p<0.005$ and vegetables, $t(76)=7.68$ $p<0.005$. The lowest scoring food group was fruits with a mean score of 1.8(SD=1.97).

Only 61% and 45% of adolescents achieved the fat ($\leq$20% of total EI) and saturated fat goals ($\leq$7% of total EI), respectively, from the moderation category. Cholesterol intake $\leq$300mg/d was observed in 75% of adolescents and 69% had sodium intake of $\leq$2400mg/d. Additionally, 70% of adolescents consumed empty calories that made up over 10% of their total daily energy intake. The balance for energy yielding nutrients as well as fatty acids was also poor. None of the adolescents met the macronutrient (carbohydrate: protein: fat) ratio recommended for a balanced diet.

Table 3. DQI-I components, the score range and the mean and standard deviation for these components.

Component	**Score range**	**Mean (Standard deviation)**	**Percentage**
DQI-I total	0-100	59.9(7.4)	59.9%
Variety	0-20	12.8(4.3)	64%
Variety (overall food group)	0-15	10.1(2.9)	67.3%
Variety (protein)	0-5	2.7(1.7)	54%
Adequacy	0-40	28.5(5.1)	71.25%
Vegetable	0-5	3.6(1.6)	72%
Fruits	0-5	1.8(1.97)	36%
Grains	0-5	4.2(0.98)	84%

Component	Score range	Mean (Standard deviation)	Percentage
Fibre	0-5	3.3(0.7)	66%
Protein	0-5	4.1(1.0)	82%
Iron	0-5	3.3(0.75)	66%
Calcium	0-5	3.4(0.8)	68%
Vitamin C	0-5	4.8(0.65)	96%
Moderation	0-30	18.5(7.8)	61.6%
Total Fat	0-6	4.4(2.16)	73.3%
Saturated fat	0-6	3.5(2.5)	58.3%
Cholesterol	0-6	5.1(1.8)	85%
Sodium	0-6	4.7(2.1)	78.3%
Empty calorie foods	0-6	0.95(1.5)	15.8%
Overall Balance	0-10	0.1(0.6)	1%
Macronutrient ratio	0-6	0(0)	0%
Fatty acid ratio	0-4	0.1(0.55)	2.5%

Multiple linear regression was carried out to determine the effects of adequacy, moderation, variety, and balance in a diet on MFQ scores in young people. The model was not significant $F(4,75)=0.82$ $p=0.52$ and explained 1% of the variance. The analysis suggests that adequacy was the most influential predictor ($\beta=0.25$) and variety being the least influential predictor ($\beta=-0.16$). However, none of the predictors significantly influence MFQ scores.

4.4 Discussion

This pilot study explored the effects of diet quality on depressive symptoms in a UK community sample of young people. Diet quality was assessed using DQI-I which included adequacy, moderation, variety, and balance in diet. Overall, there was no significant association between the diet quality components and depressive symptoms. Only one other study exploring the association of diet quality with internalising disorders showed no significant association between depression and overall diet quality. However, they did report that greater variety and adequacy of the diet was significantly associated with a lower level of emotional problems [35]. Overall, these results are in contradiction to the few previous studies that used DQI-I to assess diet quality and its association with depressive symptoms. An adult study demonstrated that DQI-I was inversely associated with depressive symptoms over a 6 year follow up period [36]. Another study conducted in Canadian children, showed that diet quality, measured using DQI-I, was inversely associated with children's feelings of worry

sadness and unhappiness [37]. Diet variety and adequacy were the components shown to be significantly associated with lower odds of the feeling of worry, sadness, and unhappiness [37]. A recent review of 21 cross sectional studies also showed an inverse association between quality diet, however, these were assessed using Healthy Eating Index and other diet quality indices [31]. These inconsistencies might be due to the differences in age group between this study and the previous studies. Changes in lifestyle behaviours such as meal practices and sedentary behaviour are common during adolescence and all affect the diet quality [38]. Another plausible explanation would be the limitation of the present study, which is the small sample size, particularly with respect to the number of participants reporting with low mood.

The mean DQI-I scores in this sample of young people was 59.9% which is in line with the mean scores reported in previous studies in the USA, China (>20 years old) [34] and southern Spain (6-18 years old) [39], but higher than the means observed in the Balearic Islands population [40]. Adequacy and variety were the highest scoring components, which was also compatible with previous studies [39-41]. According to the criteria of Kim et al [34], a score below 60% indicates a poor diet quality, suggesting that this community sample had an average diet quality. Variety in diet was assessed as overall variety and variety within protein sources. According to the DQI-I, a diet that includes a variety of protein sources is a component of a good quality diet [34]. However it is important to consider the changes in dietary patterns in the adolescent population [42], and the lower score of variety in protein may reflect these changes in traditional protein sources. Adequacy reflected compliance with daily recommendations essential to ensure a healthy diet. Even though, overall, the diet of the participants scored highly on this component for adequate intake of vitamin C, calcium, iron, protein, fibre, grains and vegetable, participants scored poorly for intake of fruits. This is again compatible with a previous study in adolescents from southern Spain where participants scored highly for intake of protein, iron, and calcium but not adequately consuming fruits, vegetables, grains, and fibres [39]. Further, in contrast to previous studies, adolescents' diet in this study was well moderated but highly unbalanced. The lowest scoring component for moderation was the consumption of empty calorie foods with 70% of the participants having over 10% of total energy due to consumption of these food items. Additionally, the overall balance defined as proportionally in the energy sources and fatty acid consumption was extremely poor and highly unbalanced. The suggested carbohydrate to protein to fat ratio was not achieved by any participant, and the recommended MUFA and PUFA ratios from total saturated fatty acids was only met by few individuals.

The fact that healthy individuals did not meet the daily recommended amount of these nutrients and the balance between them, suggests that longitudinal studies are required to

investigate if, over time, the nutritional intake doesn't meet the recommended amount, does the MFQ score change resulting in development of depression. There was also a lack of studies that directly compared the nutritional profile of young people who report clinically significant symptoms of depression and those who do not report symptoms of depression. Another factor that should be considered is the ability of individuals to absorb these nutrients. One may be consuming higher amounts of a certain nutrient doesn't necessary translate into the body absorbing the same amount, hence analysis of the blood profiles of these nutrients or their metabolites may be a more reliable measure of the amount of these nutrients as compared to a self-report. Another limitation is that, the measure used to assess the daily nutritional intake, FFQ, is memory dependent and therefore quantification of food intake would be imprecise due to poor recall or use of standard portion size, not taking into account the between person portions size differences. Additionally, social desirability effect cannot be ruled out as there are increased chances of participants overestimating the intake of foods considered healthy and under reporting the consumption of less healthy food items. Further, as demonstrated in a previous literature review [29], studies of high quality, used diet quality as a measure for nutrients, however, there are still limitations to this approach. Using food recall or a food frequency questionnaire to calculate diet quality again relies heavily on participants memory and knowledge of portion size. Further, diet quality is an imprecise term due to the heterogeneous and multidimensional nature of the concept itself. Its definition should ideally reflect aspects from a relevant number of fields such as toxicology, nutrition, economics, and the food industry and, more comprehensive and holistic measures of assessing nutritional intake are required.

The significantly low consumption of fruit is of interest as they are rich in most of the micronutrients e.g. vitamins and minerals that those with depression seem to lack [43]. Substantial evidence exists regarding the positive effects of high fruit and vegetable diet on brain development and common mental disorders including depression [44-47]. Furthermore, research suggests that a dose-response relationship exists, whereby each serving increase in fruit and vegetables is associated with an improvement in mental health [48,49]. The effects of a variety of micronutrients in these fruit and vegetable on mood have been investigated, however flavonoids have not been given enough attention. It would be of great interest to investigate the effects of flavonoids on mood as they are present in abundance in fruit and vegetables and may be an additional mediator of this association between healthy diet and depression. Additionally, traditional analyses in nutritional epidemiology, including this study, mainly investigates the association between depression and lack or presence of single or multiple nutrients or foods. Though valuable in furthering the knowledge of effects of

single nutrients on low mood, it has several conceptual limitations. Firstly, people do not consume individual nutrients. Food consists of various combinations of nutrients which may have alleviating or exacerbating effects. Secondly, the effect of a single nutrient may be exceptionally low and difficult to measure, while the collective effects of some nutrients in a dietary pattern are easily detectable. Therefore, it is as important to explore the effects of various diet patterns on depressive symptoms especially in young adults.

4.5 Conclusion

In conclusion, this exploratory study showed that there was no significant association between diet quality and depressive symptoms in a UK community sample of adolescents. Overall, adolescents do not meet the daily recommended amounts of fruit and were shown not to have a balanced diet regarding the ratio of carbohydrate to protein to fat. In addition to replicating this study in a larger group of adolescents, further studies should include longitudinal designed studies to explore whether healthy adolescents that do not meet the recommended daily dietary requirements develop symptoms of depression later in life. Exploring all the micronutrients abundantly present in fruit and vegetables such as flavonoids may also be of potential in explaining the link between healthy diets and low incidence of depression.

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Chapter 5: Introduction to Flavonoids

5.1 Introduction

Flavonoids are a large subgroup of polyphenols and are the main reason why polyphenolic compounds are of importance. Polyphenols are phytochemicals found abundantly in plants and are involved in important functions such as attracting pollinating insects, regulating cell growth, and combating microbial infections. They are known to have antioxidant properties and play a potential role in prevention of various diseases such as cancer, cardiovascular and neurodegenerative diseases [(1)]. Furthermore, polyphenols are thought to modulate the activity of several cell receptors and enzymes resulting in their influence on other biological actions that are not yet fully understood [(2)].

Flavonoids can be found in varying concentrations in plant-based food such as berries, tea, cocoa, soybeans, and grains. Leaf, bark, and berries of such plants are commonly used to prepare herbal extracts as a more concentrated source of flavonoids. Their pigment can range from red, purple, and blue depending on their pH. Flavonoids can be further sub categorised into 12 classes based on their molecular structure, of which six are of dietary significance. All flavonoids have the same common structure of two benzene rings (indicated as A and B), as shown in Figure 1, linked via a heterocyclic pyrene ring (labelled C). The extent of oxidation, substitution of C-3 position and/or hydroxylation pattern of the C ring determines the class of flavonoid [(3)]. Table 1 shows the structures of the subclasses of flavonoid with dietary significance and the food sources they are present in.

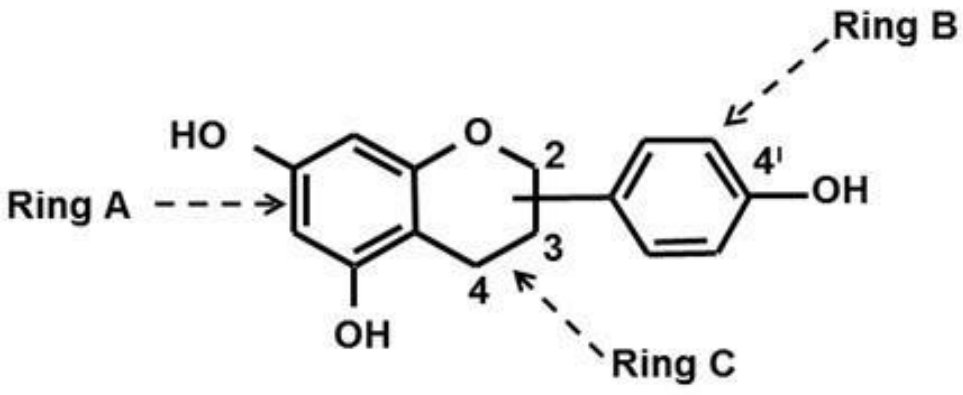
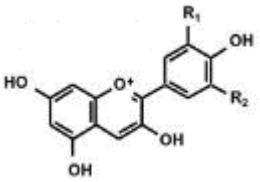
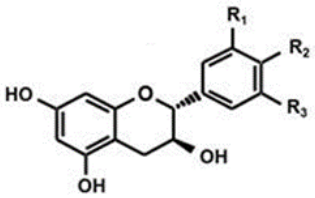
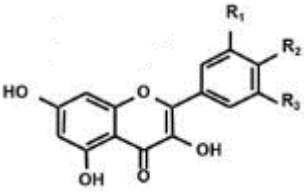
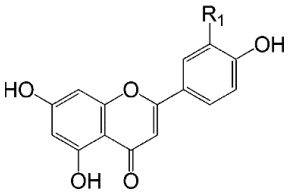
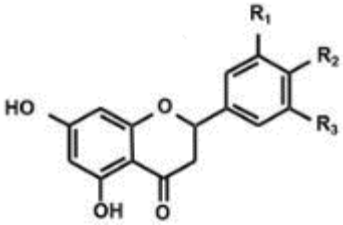
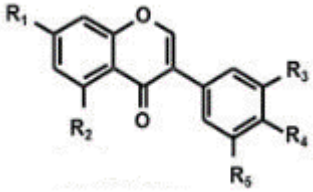


Figure 1. showing the general flavonoid structure, adapted from (Spencer et al., 2008).

Table 1. Flavonoid subclass, their naturally occurring form, and structures.

Flavonoid Subclass	Food source	Structure
Anthocyanidins **e.g. Cyanidin** **delphinidin**	Berries e.g. blueberries, black currants, strawberries, grapes. Aubergines and red cabbage.	
Flavanols **e.g. catechin**	Tea e.g. green and black cocoa (dark chocolate not necessarily in milk chocolate confectionaries). Fruits like apricots and apples	
Flavonols **e.g. kaempferol, quercetin**	Vegetables e.g. onions, curly kale, and leeks Small quantities in fruits	
Flavones **e.g. apigenin, luteolin**	Cereals Herbs like parsley Small amounts in celery	
Flavanones **e.g. naringenin**	Citrus Fruits e.g. orange, grapefruit, and lemons	
Isoflavones **e.g. daidzein, genistein**	Soya Beans, soy flour, tofu, soymilk Peanuts	

5.2 Flavonoids derived general health benefits

There has been increasing interest in the health benefits of food rich in flavonoids. Epidemiological and clinical studies suggest that increased levels of dietary flavonoids can play a protective role against several diseases such as cardiovascular disease (CVD), stroke, diabetes, neurodegenerative disease, and cancer.

Based on several in vitro and in vivo studies flavonoids have shown considerable promise in reducing the risk of cardiovascular heart diseases by inhibiting low-density lipoprotein cholesterol oxidation [4,5] reducing platelet aggregation [6] ischemic damage [7] and oestrogen like activity [8]. Studies show that flavonoid intake is also inversely correlated to plasma cholesterol levels which also is a contributor to heart diseases [9,10]. Chronic cocoa, tea flavanol and blueberry anthocyanin interventions are thought to lead to nitric oxide triggered vasodilation which results in positive effects on endothelial functions mainly linked to vascular health [11-15]. Interventions with flavanol-rich chocolate for 2 to 18 weeks, have also shown to have a positive effect on blood pressure [16,17]. Reactive oxygen species formed through lipid peroxidation, oxidizes the amino acid residues of low-density lipoprotein, elevation of which is a primary risk factor for atherosclerosis and coronary artery disease.

Epidemiological studies have also found an inverse relationship between the amount of fruit and vegetable consumed and incidence rates of various types of cancer. However, the evidence of this relationship is not comparable between studies, as concluded in a study reviewing the effects of dietary polyphenols in human health [18]. Invitro studies have reported a positive effect of flavonoids on retardation of tumour development. Clinical studies, however, have not been as conclusive [18,19]. Another review concluded that although the epidemiological evidence to support cancer protective effects of flavonoids are limited and inconsistent, there is an inverse associations between soy intake, early in life, and premenopausal breast cancer, green tea consumption and stomach cancer, onion and apple consumption and lung cancer [20].

The mechanisms by which flavonoids exhibit these potential cancer protective effects may be due to their neuroprotective and anti-oxidative effects. Flavonoids inhibiting oxidation and protect the body from harmful effects of free radicals. Free radicals are usually oxygen molecules that have lost an electron and in doing so become reactive and unstable and can cause harmful effects. For example, lipid peroxidation can result in cell membrane damage resulting in shift in the net charge of the cell causing osmotic pressure leading to swelling and eventually cell death. Inflammation, coronary heart diseases, diabetes, arthritis, Alzheimer's, and cancer have all been linked to free radicles. These free radicals are formed in the body

due to energy production, stress- hormones such as cortisol and catecholamines degeneration. External factors such as processed foods containing high levels of lipid peroxides, cigarette smoke, air pollution, alcohol and electromagnetic radiation including sunlight also contribute to the production of free radicals. Due to this the body's natural antioxidants may not be sufficient to neutralise the free radicals produced. Therefore, supplementing antioxidants via diet may prove beneficial. DNA oxidation and damage has also been correlated with risk of cancer. Numerous studies have demonstrated protective effects of flavonoids where a study found a reduction in oxidative DNA damage in men who consumed 700 ml of a high anthocyanin red berry juice over the 4 weeks when compared to a control group [20].

Inflammation plays a role in neurodegeneration. The enzyme Cyclooxygenase (COX) facilitates inflammatory response by playing a role in the production of arachidonic acid a precursor to compounds responsible for inflammation. Selected flavonoids like quercetin have shown to inhibit cyclooxygenase pathway. Another way arachidonic acid is produced is by degradation of immune cells such as neutrophils. Flavonoids are also shown to have the ability to inhibit this degranulation and thereby preventing inflammation [21,22].

5.3 Flavonoids derived Cognitive benefits

The beneficial effect of flavonoids on cognition in humans has only very recently been of interest. Several chronic and acute studies have investigated the effects of variety of dietary flavonoids on cognition across a range of age groups. In this section evidence from intervention studies using flavonoids from food items commonly found in diet to explore its effects on cognition will be discussed.

5.3.1 Cocoa Flavonoids (Epicatechin): Most of the research literature examining the effect of flavonoids on cognition has focused on the effects of cocoa, which is rich in the flavanol epicatechin, and contains caffeine and theobromine, which have psychoactive properties. An acute, double-blind, crossover study, in 30 young adults, compared two doses of chocolate milk with a fully matched control to investigate its effects on cognitively fatiguing tasks. Improvements in working memory on the least demanding tasks were reported after the consumption of 520mg of cocoa flavanol. The high dose of 994mg resulted in improvement in attention tasks. Overall, the low dose was observed to be effective over a wider range of time points [23]. Similarly, another study reported an improvement in the visual spatial working memory and reaction times in 30 young adults aged 18-30 years, after the consumption of 773mg dose of coca flavanols when compared with low flavanol white chocolate control [24]. However, levels of caffeine and theobromine were not matched making

it difficult to conclude if the effects were flavonoid derived. Another acute between-subjects intervention reported an improvement in working memory from baseline after the consumption of 250mg coca flavanol in 40 young adults. However, the control intervention was not matched for caffeine. In contrast, a similar acute study in older adults failed to observe any effects of 250mg and 500mg of cocoa, on cognition, assessed using a broad spectrum of cognitive tasks [25]. Chronic studies where healthy older adults consumed chocolate bars for 6 weeks and a 5-day chocolate drink intervention in younger adults also showed no effects of cocoa flavanols on cognition [26,27]. Overall evidence suggests that flavonoids rich cocoa may have an acute effect on attention, working and visuospatial memory, in adults, however no long-term beneficial effects on cognition were observed.

5.3.2 Apple (Quercetin & Epicattechin): Few studies have investigated the effects of apple flavonoids on cognition. A crossover intervention study investigated the acute effects of whole fresh apples (184mg quercetin & 180mg epicatechin), alone and in combination with spinach (for its nitrate content) on cognition in 30 middle aged participants. The study failed to show any significant improvement in cognitive tasks with apple on its own and in combination with spinach [28]. As suggested in a recent review, this lack of effect may be due to the optimal time for observing the benefits of apple flavonoid on cognition was missed or because the flavonoids present in apples are not particularly effective [29]. More studies using different does of apple flavonoids and measuring cognition at different time intervals are required to come to an accurate conclusion about the effect of apple flavonoids on cognitive functioning.

5.3.3 Citrus fruits (citrus hesperdin): Citrus fruits contain several flavonoids with the most common being the flavanone hesperidin. Similar to apple flavonoids, studies exploring the cognitive effects of citrus flavonoids is limited with only orange used as the source. In an acute crossover intervention study, flavonoid rich orange juice containing 70.5mg of total flavonoids, 42.15mg of hesperidin, 6.75mg narirutin and 17.25mg naringin, or a matched control was used to supplement 24 young adults. Executive functioning, processing speed, vision, episodic and working memory were assessed at baseline and 2 hours postprandially. Significant improvements in working memory and psychomotor speed were reported after the consumption of orange juice when compared to baseline and control drink [30]. Another study using a greater flavonoid content (272mg; 220.46mg hesperidin, 34.54mg narirutin) by adding flavanone-rich orange pulp to the fortified juice also observed improved psychomotor performance, attention, and general executive function in middle aged adults. Overall, it appears that orange juice benefits psychomotor performance in adults. However, the research on citrus flavonoids are limited.

5.3.4 Berry anthocyanins (Blackcurrant, blueberry, cherry, cranberry, grape): Most of the studies investigating the effects of flavonoids on cognition have used berries as the flavonoid source. Evidence shows acute and chronic benefits of berry flavonoids consumption in improving cognitive functioning. A study carried out in 19 young adults demonstrated an improvement in working memory, 5 hours following the consumption of freeze-dried whole blueberries containing 631mg of anthocyanidins [(31)]. A later study using fresh blueberries containing 143g of anthocyanins found no significant effects on executive function but observed significant improvement in delayed word recall in 14 children [(32)]. The authors conducted a larger double-blind placebo controlled, cross over study in 21children. They investigated the effects of blueberry flavonoids, two sperate doses of 127mg and 253mg anthocyanins, on cognitive functioning. The highest does resulted in significant improvements in immediate word recall 1.25 hours post intervention and in delayed word recognition after 6 hours. Accuracy was also improved 3 hours post intervention for cognitively demanding incongruent trials [(33)]. Evidence showing the effects of chronic blueberry intervention is limited with one study of 12-week intervention of blueberry juice in older adults with mild cognitive impairments. The chronic study suggested that the carbohydrate to protein to fat ratio in diet juice intervention resulted in improved immediate verbal and spatial memory. However, the small sample size (9 participants) and the four-year mean age different between the intervention and blueberry group, those in blueberry group being younger, means the results must be interpreted with caution.

Blackcurrants are also a rich source of anthocyanins. In a double-blind, controlled crossover study, blackcurrant as cold pressed juice or freeze-dried powder were used as flavonoid intervention. In 36 young adults, blackcurrant intervention resulted in improved attention during a cognitively fatiguing tasks 1-hour post intervention when compared to the placebo control [(34)]. There is a lack of acute and chronic studies in the literature using blackcurrants to come to a definite conclusion about its effects on cognition across different age groups.

Grape juice has also been used to investigate flavonoid benefits on cognition. In an acute double-blinded cross over study, the effects of grape juice containing 580mg of anthocyanins or a match controlled on deficits in cognition that occurs post large meal consumption was explored. No significant effects of grape juice on implicit memory were observed in 35 smokers recruited, 1 hour postprandially when compared to the energy matched control [(35)]. In contrast, a study using purple grape juice reported a significant cognitive effect 20 minute postprandially when compared to sugar matched white grape juice [(36)].

The effects of cherry juice have also been explored. Consumption of cherry juice containing 55mg anthocyanins by 6 younger adults and 5 older adults with mild cognitive impairments resulted in improvement in working memory in older adults 6 hours post intervention. No other cognitive benefits were observed, and the author attributed the significant result to type 1 error [37]. The small sample size also suggests the study to be underpowered and the absence of an energy matched low flavonoid placebo condition is also a major limitation of the study.

Overall research using flavonoids to explore their effects on cognition is limited, especially chronic studies. Those that exist are difficult to compare due to lack of consistency in the type of flavonoid used, the age group recruited, and the cognitive domains targeted.

5.4 Flavonoids, cognition, and mood

The association between mood disorders such as depression and cognitive impairment is well documented. These impairments have been identified across several cognitive domains for example executive functioning, episodic memory, semantic memory, visuo-spatial memory, and information processing speed [38-44]. Depressed individuals have difficulties with memory tasks such as list learning and free recall. Short term memory is thought to be impaired with studies reporting a decreased ability to acquire and retain new information, perhaps because depression is also associated with high levels of intrusive, rumination [45-48]. Impairments in verbal learning and difficulty in transferring information from short term to long term storage has also been reported in both unipolar and bipolar depression [49,50]. Reaction time also seems to be impaired in individuals with depression [51,52]. Reaction time also deteriorates much more quickly in depressed individuals compared to healthy individuals [53]. The extent to which cognitive problems in depression are due to fatigue, low motivation or psychomotor retardation is not known.

The most common cognitive impairment reported during depression is associated with executive functions. Those suffering from depression show difficulty in hypothesis testing and cognitive flexibility and brain imaging studies suggest that reduced blood flow to the medial prefrontal cortex and dorsal anterior cingulate cortex is consistent with executive functioning impairments in depressed individuals [54]. Interestingly, a correlation between depression and severity and magnitude of cognitive defects have been reported in individuals with major depressive disorders and these deficits disappear after clinical remission [55-57]. However, some studies suggest that cognitive deficits, especially executive functioning, verbal learning, and memory, persist among young adults after remission and can represent a trait marker of the disorder. Further studies are required to better explore the cognitive

impairments in low mood but there is enough evidence to suggest a consistent association between depression and cognitive functioning. Given this well-established, yet poorly understood relationship between cognitive impairment and depression, and the impact of dietary flavonoids on cognitive performance, it can be hypothesised that dietary flavonoids may have an indirect influence on mood. However, studies investigating the effects of these dietary flavonoids on mood are even more rare. Only a few studies of the effects of flavonoid on cognition have also included a transient mood measure [23,25,28]. One study reported a significant improvement in self-reported mental fatigue and another study reported a decrease in anxiety after the flavonoid treatment. There are several methodological limitations, from the duration of the intervention to the tools used to assess transient mood and its crucial to investigate the effects of acute and chronic dietary flavonoids on transient and long term mood especially in adolescent and young adults as it is a critical time for brain development.

5.5 Conclusion

In conclusion, the evidence suggests that research into cognitive benefits of flavonoid rich foods is a promising area requiring further investigation. Existing evidence also demonstrates an association between depression and cognition, where defects in executive functioning is correlated to existence and severity of depression. These cognitive benefits of flavonoids rich foods therefore can be hypothesised to improve overall mood. Taking into account that there may be an underlying effect of flavonoids consumption on mood and the limited number of studies in the literature exploring this link, the next series of studies in this thesis will attempt to investigate the effects of acute and chronic wild blueberry flavonoid consumption on depressive symptoms and cognition.

5.5 References

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Chapter 6: Study 3: Effects of Acute Blueberry Flavonoids on Mood in Children and Young Adults

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Measures included:
Positive And Negative Affect Schedule (PANAS) – Appendix 5.

Effects of Acute Blueberry Flavonoids on Mood in Children and Young Adults

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Abstract

Epidemiological evidence suggests that consumption of flavonoids (usually via fruits and vegetables) is associated with decreased risk of developing depression. One plausible explanation for this association is the well-documented beneficial effects of flavonoids on executive function (EF). Impaired EF is linked to cognitive processes (e.g., rumination) that maintain depression and low mood; therefore, improved EF may reduce depressionogenic cognitive processes and improve mood. Study 1: 21 young adults (18–21 years old) consumed a flavonoid-rich blueberry drink and a matched placebo in a counterbalanced cross-over design. Study 2: 50 children (7 to 10 years old) were randomly assigned to a flavonoid-rich blueberry drink or a matched placebo. In both studies participants and researchers were blind to the experimental condition and mood was assessed using the Positive and Negative Affect Schedule before and 2 h after consumption of drinks. In both studies the blueberry intervention increased positive affect (significant drink by session interaction) but had no effect on negative affect. This observed effect of flavonoids on positive affect in two independent samples is potentially of practical value in improving public health. If the effect of flavonoids on positive affect is replicated, further investigation will be needed to identify the mechanisms that link flavonoid interventions with improved positive mood.

Keywords: depression; mood; affect; dysphoria; cognition; flavonoid; blueberries; children; young adults

6.1 Introduction

Major depressive disorder is the leading international cause of disability and is estimated to affect 350 million people worldwide [1]. It is the second most common cause of death in 15- to 29-year-olds, via suicide [1]. Current treatments for depression include psychological therapies and a range of pharmacological agents. The treatment options recommended for children and adolescents are limited, with only one recommended pharmacological treatment, fluoxetine, in addition to psychotherapy. These treatment options are further constrained because of concerns about the use of anti-depressant medication within young people and because most young people do not have easy access to psychological therapies [2–4]. Therefore, there is a pressing need for alternative interventions, especially those that offer a cost-effective and practical means of preventing, or alleviating, depression in this population.

A common symptom of depression is impaired cognitive functioning, with significant deficits in executive functioning (EF). EF is an umbrella term, describing cognitive processes such as working memory, planning, problem-solving, cognitive flexibility, inhibitory control, directing attention, thoughts and, therefore, behaviours. Impaired EF is believed to maintain depressive symptoms, such as negative self-perception and low mood, via perseveration and rumination [5–8]. Importantly, EF is associated with the development of the frontal area of the brain, an area that continues to mature and develop throughout adolescence and into early adulthood [9,10]. Thus, any disturbance to the development of the frontal region during this critical period (for example because of an episode of depression) can have a long-lasting impact and may explain why depression that occurs during adolescence and early adulthood is associated with long-term impairments into adult life [11,12].

Flavonoids are a class of polyphenols (micronutrients) found naturally in fruits, vegetables, tea, coffee, and cocoa. Flavonoid consumption has been associated with both vascular and cognitive benefits across the lifespan [13–17]. Single-dose flavonoid interventions have produced improvements in attention, inhibition, visuospatial memory, and executive function between 2–6 h post-consumption [14,18–20], whilst supplementation of flavonoids for 1.5 to 8 weeks has been associated with improved visuospatial memory and improved long-term memory [15, 21,22]. Numerous mechanisms of action have been investigated to explain the beneficial effects of flavonoids on cognition. These include increases in cerebral blood flow, protecting against neuronal stress via anti-inflammatory and anti-oxidative effects, and positively stimulating neural signaling pathways, such as Extracellular Signal Regulated Kinase (ERK), Serine/Threonine-specific Protein Kinase (Akt) and Brain-Derived Neurotrophic Factor (BDNF), leading to improved neural signaling [15, 21, 23,24].

Independently of the experimental evidence that shows flavonoids improve cognitive performance, there is emerging evidence that flavonoids may also support mental health and well-being. Epidemiological data shows that lifetime consumption of fruit and vegetables (and therefore higher flavonoid consumption) predicts a lower incidence of depression in later life [25–29]. The benefits are also seen earlier in life; a recent systematic review concluded that whilst the quality of evidence was weak, there was a consistent body of research reporting cross-sectional and longitudinal associations between nutrition and mental health in children and young people [30]. Similar findings have been shown by other authors [31,32]. However, there is an absence of studies exploring the effects of flavonoid-rich interventions on mood.

Given the well-documented links between flavonoid consumption and cognition, and between cognition and depression, the studies reported in this paper assess the acute effects of flavonoid-rich wild blueberries (WBB) on mood two hours post-consumption. This two-hour interval coincides with the timeframe for the peak absorption and metabolism of the anthocyanins present in blueberries [33]. In addition, it is important to establish whether acute effects on mood are observable prior to considering a chronic flavonoid-based intervention for mood outcomes. Two independent groups, healthy children, and young adults were recruited. These groups represent individuals who are at crucial stages of mental and cognitive development and thus plausible points at which prevention and public health interventions may be particularly powerful.

6.2 Methods

6.2.1 Ethics: The research was reviewed and given a favorable ethical opinion for conduct by the University of Reading Research Ethics Committee (2015-148-CW & UREC 15/10) and was conducted in accordance with the Declaration of Helsinki. All participants were screened for food related allergies or other health conditions, e.g., diabetes, heart disease, blood pressure, thyroid, kidney, and liver diseases which would exclude them from the study.

6.2.2 Study 1 (Young Adults)

6.2.2.1 Participants: 21 undergraduate students were recruited from University of Reading; 19 females and two males aged between 18 and 21 years (Mean (M) = 20.14 years, Standard Deviation (SD) = 1.01).

6.2.2.2 Drink preparation and consumption: All interventions were prepared on site, no more than 20 minutes before consumption, by an independent researcher who did not

administer the drink to participants. The flavonoid-rich wild blueberry (WBB) drink contained 253mg anthocyanins and was prepared by mixing 30g of freeze dried WBB with 30mL of low-flavonoid Rocks Orange Squash and 220mL of water. The placebo drink was matched to the WBB drink for vitamin C (4mg), sugars (8.90g) fructose, (7.99g glucose), 30mL Rocks Orange Squash (containing 13.2mg total polyphenols Narirutin & Hesperidin) and 220mL of water. The drinks were 116.6kcal. Analysis of anthocyanin content was carried out by independent researchers from the University of Reading using the methods described in Rodriguez-Mateos et al .,2012[34] indicating anthocyanin content of 8.43 mg/g which, given a freeze dried to fresh ratio of 7/1, is equivalent to 120.5 mg/100g fresh (see Table 1). Drinks were prepared in an opaque cup and straw to ensure that double blinding was maintained.

Table 1. Showing the anthocyanin content of the intervention drink.

	mg/g freeze dried	Stdev	mg/ 100 g fresh BB	Stdev
Delphinidin	3.29	0.21	46.94	3.03
Cyanidin	1.17	0.07	16.64	0.95
Petunidin	1.58	0.08	22.50	1.16
Peonidin	0.37	0.02	5.24	0.30
Malvidin	2.04	0.10	29.14	1.50
Total	**8.43**	**0.48**	**120.47**	**6.92**

6.2.2.3 Mood Measure: The Positive and Negative Affect Schedule-NOW (PANAS-NOW) was used to assess current mood. The PANAS-NOW is a valid and reliable 20-item (10 positive and 10 negative mood states) self-report measure of Positive Affect (PA) and Negative Affect (NA) [35,36] which can be used on multiple test occasions. Participants were asked to rate the degree to which they were currently experiencing each item, on a five-point Likert scale. The ratings of positive and negative items were summed to calculate an overall positive and overall negative affect score, ranging from 10–50 (lower scores indicating lower levels of positive or negative affect). A cognitive performance assessment (Modified Attention Network Task; MANT) was also administered after each administration of the PANAS, the data for which will be reported elsewhere.

6.2.2.3 Procedure: This was a double-blind, placebo-controlled, crossover study. Informed consent was obtained from all participants for inclusion before they participated in the study. To minimise variability caused by prior flavonoid consumption, participants were given a list of high polyphenol food items (such as tea, coffee, chocolates, most fruits and vegetables) and asked not to consume these for 24 h before each test session including the morning of the sessions. All test sessions were scheduled in the morning and all participants attended three

test sessions separated by a minimum three-day wash-out period (range three to seven days; median three days). This included a practice test day (Screening), which was described to the participants as the first test day, where all participants received the placebo drink. This was to ensure participants were fully versed in the experimental procedures before testing began. Thereafter they were tested on two further occasions, during which the placebo or flavonoid intervention was administered to each individual in a random order (10 received WBB first).

On each of the three visits to the laboratory, participants completed the baseline mood measure (PANAS-NOW) on arrival. Immediately following this they consumed the drink. They were asked to return to the laboratory 2 h later and refrain from eating, exercising, or drinking during this time. However, water consumption was permitted before and during the 2 h break. Upon their return, participants completed the PANAS again. Overall, each visit to the laboratory lasted approximately 30 min.

6.2.3 Study 2 (Children)

6.2.3.1 Participants: 52 participants (29 female) aged seven to ten years (M = 8.241, SD = 0.965) were recruited from two local primary schools in Berkshire, UK.

6.2.3.2 Drink preparation and consumption: On the test day, participants were randomly allocated to receive the wild blueberry drink (*n* = 28; 17 females; Mean age = 8.236, SD = 0.869) or a matched placebo (*n* = 24; 12 females; Mean age = 8.227, SD = 1.031). The contents of each drink were identical to Study 1, but instead only included 170 mL of water to aid consumption and palatability in a child population. A confederate prepared all drinks at the school immediately before they were administered to the participants by the researcher. Drinks were offered in an opaque drinking cup with a black straw placed through the lid to ensure double blinding.

6.2.3.3 Measures: A computerised version of the children's Raven's Coloured Progressive Matrices (RCPM) was administered at screening to measure fluid intelligence. This is a well validated measure assessing nonverbal and reasoning abilities [(37)]. This measure was included to ensure all participants were of healthy cognitive functioning for their age, and to ascertain intervention effects in relation to intelligence. The York Assessment of Reading Comprehension was administered to ascertain whether participants were at age-appropriate reading comprehension levels. A modified Continuous Performance Task was also employed to assess possible attentional deficits. Data from these two tasks were not used in the analysis of the current study. A practice of the PANAS-C and cognitive task battery was also

undertaken to ensure understanding of the tasks and reduce practice effects. This practice data was not analysed.

For the main test sessions, the Positive and Negative Affect Schedule for Children (PANAS-C) was administered alongside a 40 min cognitive battery which included Rey's Auditory Verbal Learning Task (RAVLT), Modified Attention Network Task (MANT) and Test of Word Reading Efficiency (TOWRE-2) [38–40]. The cognitive performance data is reported elsewhere [41].

PA and NA were calculated using the validated children's version of the PANAS; PANAS-C [37]. The PANAS-C has 30 items (15 positive and 15 negative emotions) and includes the original 20 items from PANAS-NOW and 10 additional child-friendly synonym items derived from the PANAS-X (Expanded Form). This was administered and analysed as in study one.

6.2.3.4 Procedure: This study was also double-blind, and placebo controlled. A between-groups design was used to minimise disruption to the school and the demands on participants. All children took part in a screening session one to two days before the main test day. The main test day consisted of a baseline session and a post-consumption session 2 h later. All parents or legal guardians gave written consent for their child to take part and each child gave verbal assent before any research began. Parents confirmed that their child had no allergies or food intolerances that would prevent them from taking part. To accommodate school hours, children were tested during the afternoon at school. They were not required to fast prior to testing. However, parents were asked to make sure that their child consumed a low-flavonoid diet for 24 h before the baseline session, including breakfast and lunch on the main test day. Parents were telephoned after their child's screening session to give them the date of their child's next test day and to remind them about the dietary restrictions. School canteen staff members were also asked to monitor children's meals on the day of testing and to remind the children taking part in the study not to eat high-flavonoid foods for lunch that day.

Children were tested individually in a quiet space at school on two separate occasions, one to two days apart. On day one, children completed the screening and practice tasks outlined previously. On testing day 2, after a low-flavonoid lunch, participants completed the PANAS-C and cognitive battery (data reported elsewhere [41]), before consuming the placebo or flavonoid drink. They then returned to their classroom and were asked not to exercise or consume anything except water. After 2 h, participants individually completed the PANAS-C and a matched version of the cognitive battery again in the presence of the researcher.

Participants were also given a written debrief about the study aims and were given similar debrief information to take home to their parents.

6.2.4 Analysis: Data was analysed using SPSS (Version 22.0). In both studies PA and NA were dependent variables in a two-way analysis of variance (ANOVA) with Drink (Placebo, WBB) and Session (pre-and post-consumption) as independent variables. For study 1, this was a fully repeated measures 2x2 ANOVA (as participant consumed both drinks and were tested at all time points), and for study 2 this was a mixed 2x2 ANOVA as participants consumed either the Placebo or WBB, and were test at both time points. Significant main effects and interactions were explored with Bonferroni corrected post-hoc t-tests. Baseline differences in mood (study 1 and 2) and Intelligence Quotient (IQ) (study 2) were examined using one-way ANOVAs with Drink (placebo and WBB) as the independent variable.

6.3 Results

6.3.1. Study 1 (Young Adults)

One-way ANOVA was performed on baseline mood scores, revealing no significant differences in mood at baseline (p>0.05). Figure 1 shows PA and NA before and after consumption of the placebo and flavonoid drinks. There was no significant main effect of Drink on positive affect ($F(1,20) = 1.24$, $p = 0.28$,). There was a significant main effect of Session ($F(1,20) = 10.67$, $p = 0.004$) and a significant Drink by Session interaction ($F(1,20) = 7.5$, $p = 0.013$,). Subsequent Bonferroni-corrected post hoc t-tests demonstrated that there was a significant increase in PA after consuming the blueberry drink (pre: $M = 22.76$, $SD = 9.01$ and post: $M = 29.48$, $SD = 8.60$; $t(20) = -4.68$, $p < 0.001$). There was no change in PA after consuming the placebo drink (pre: $M = 24.0$, $SD = 9.59$ and post: $M = 25.24$, $SD = 8.50$; $t(20) = -0.73$, $p = 0.48$, Figure 1a). Post hoc tests showed no significance difference in PA and NA between drinks at baseline ($p > 0.05$) but there was a significant difference in PA scores post-consumption between the Placebo and WBB drink ($t(20) = 2.286$, $p = 0.033$). The main effect of Session was explained by an increase in positive affect post-consumption ($M = 27.36$, $SD = 8.55$) relative to pre-consumption ($M = 23.38$, $SD = 9.3$), which, as indicated by the significant interaction, was driven by the WBB drink.

There was a significant main effect of Session on negative affect ($F(1,20) = 8.30$, $p = 0.009$,). After consuming both drinks, the placebo and the WBB, participants reported a reduction in negative affect (pre: $M = 13.17$, $SD = 2.67$ and post: $M = 12.19$, $SD = 2.49$; Figure 1b). This may be explained by the effect of the matched sugar content of both drinks. There was no

significant main effect of Drink on negative affect ($F(1,20) = 0.67$, $p = 0.42$,) and no significant Drink by Session interaction on negative affect ($F(1,20) = 0.51$, $p = 0.49$).

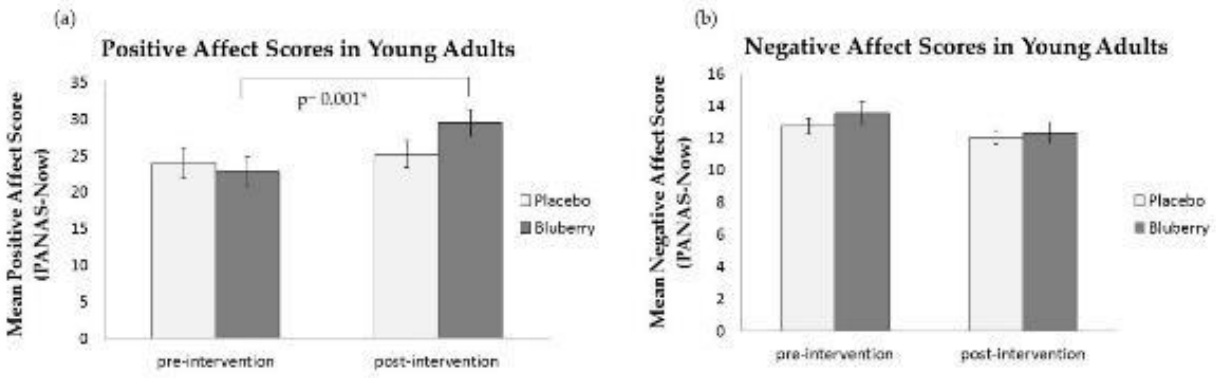

Figure 1. Mean PANAS-NOW Mood scores in adults aged 18–21 years: (**a**) Mean PA scores pre- and post-consumption of placebo and intervention drinks; (**b**) Mean NA scores pre- and post-consumption of placebo and intervention drinks.

6.3.1 Study 2 (Children)

Forty-nine participants were included in the analysis of IQ due to three missing data files. There were no significant differences at baseline between groups for IQ (Ravens: WBB group, $M = 26.78$, $SD = 4.31$; Placebo group, $M = 26.55$, $SD = 5.98$; $F(1,47) = 0.024$, $p = 0.878$), PA (WBB group, $M = 49.00$, $SD = 11.96$; Placebo group, $M = 49.21$, $SD = 10.04$; $F(1,50) = 0.005$, $p = 0.947$) or NA (WBB group, $M = 20.79$, $SD = 7.43$; Placebo group, $M = 19.25$, $SD = 3.86$; $F(1,50) = 0.832$, $p = 0.366$). One-way ANOVA revealing no significant differences in mood at baseline between the treatment groups ($p>0.05$).

Figure 2 shows PA and NA in both groups before and after the intervention. There was no significant main effect of Session ($F(1,50) = 1.362$, $p = 0.249$) or Drink ($F(1,50) = 0.456$, $p = 0.503$) for PA. However, there was a significant Session by Drink interaction ($F(1,50) = 4.176$, $p = 0.046$). Paired samples *t*-tests for each condition revealed no significant change in PA after consuming the placebo drink (pre: $M = 49.21$, $SD = 16.04$ and post: $M = 48.29$, $SD = 15.51$; $t(23) = 0.564$, $p = 0.578$), but a significant increase in PA after consuming the WBB drink (pre: $M = 49.0$, $SD = 14.86$ and post: $M = 52.36$, $SD = 14.36$; $t(27) = -2.495$, $p = 0.019$) (Figure 2a). There was no significant difference in PA between Placebo and WBB at the post-consumption time point ($t(52) = -1.597$, $p = 0.116$).

Negative affect in both groups is shown in Figure 2b. There was no significant main effect of Session ($F(1,50) = 0.009$, $p = 0.927$) or Drink ($F(1,50) = 0.355$, $p = 0.554$) on NA. There was also no significant Session by Drink interaction ($F(1,50) = 0.453$, $p = 0.504$) (Figure 2b). Negative affect did not change 2 h after consuming either the placebo (pre: $M = 19.25$, $SD =$

8.73 and post: *M* = 19.79, *SD* = 9.78) or the WBB drink (pre: *M* = 20.79, *SD* = 8.09 and post: *M* = 20.07, *SD* = 9.06).

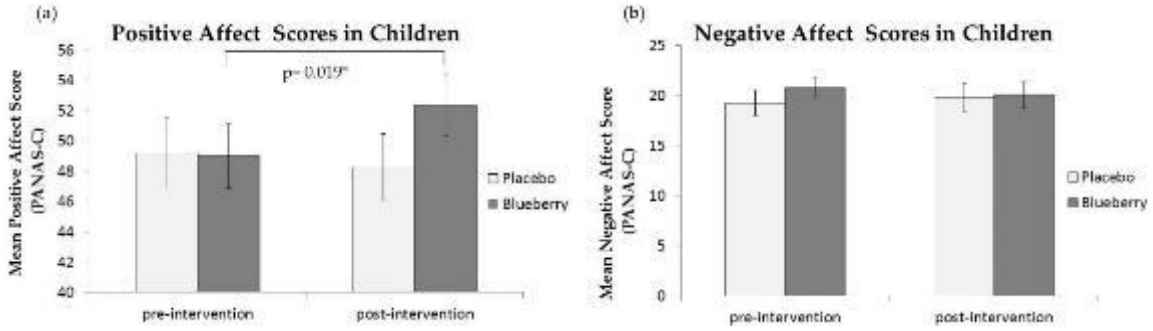

Figure 2. Mean PANAS-C Mood scores in children aged 7–10 years: (a) Mean PA scores pre- and post-consumption of placebo and intervention drinks; (b) Mean NA scores pre- and post-consumption of placebo and intervention drinks. * Significant at < 0.05. Attained from post hoc paired samples *t*-test.

6.4 Discussion

These randomised, placebo-controlled, double-blind studies investigated the effects of acute consumption of a flavonoid-rich wild blueberry drink on the mood of healthy children and young adults. In both studies, increased positive affect was observed 2 h after consumption of the flavonoid-rich drink (significant drink by session interaction). The flavonoid drink had no effect on negative affect. The effect of flavonoids on mood was consistent across two populations, at two different time points (morning and afternoon), and in a between- and a within-subject design. Thus, the positive effect of blueberry flavonoids on positive affect appears to be robust to variations in experimental design.

The distinctive effect of flavonoids on PA but not NA is notable. PA and NA reflect orthogonal facets of mood. A low PA is more highly linked to depression, and high NA is more closely related to anxiety [35-37]. Thus, these data suggest that the effect of flavonoid consumption on mood may be specific to depressive disorders, rather than pervasive across different mood states. In the study with young adults, both drinks led to a decrease in negative affect. This may be due to the sugar content of both the drinks, as dietary carbohydrates have been shown to enhance the uptake of circulating tryptophan (a precursor of serotonin) into the brain by promoting insulin secretion [42,43].

Although preliminary, these results are intriguing and warrant focused investigation of the relationship between flavonoids and mood, as well as with mental health more generally. It is important to note that diagnosis of mental health disorders or consumption of medication were not specific exclusion criteria; however, the data showed normal levels of positive and negative affect [34,44], indicating a healthy sample. The adult participants were predominantly

female, and therefore these results may not be generalised to a male population; however, there is no evidence to suggest a gender-specific mechanism underlying the effects of flavonoids on the brain. The sample of children was of average IQ and the young adult population was recruited from a university population and thus likely to have an average to above-average IQ. No carry-over effects of flavonoids are expected as the half-life of flavonoids is estimated to range from 2 to 28 h and there was a minimum of three days insured between the test days [45].

Mood is by definition a short-term experience. In non-clinical populations mood is usually labile. However, sustained periods of low mood (dysphoria) are a strong predictor of the emergence of major depressive disorder. Therefore, if acute flavonoid consumption improves positive affect, sustained consumption of flavonoids may help prevent dysphoria and thus major depression. Given that depression tends to emerge for the first-time during adolescence or early adulthood and is likely to reemerge as a relapse later in life, an intervention that increases flavonoid consumption during this critical period of development could decrease the incidence of adolescent and life-long depression.

The mechanism linking flavonoids and mood is not known and requires greater consideration. There are a number of plausible mechanisms that may explain these results. One is the finding that flavonoids increase cerebral blood flow [46]. One of the last brain structures to mature is the dorsolateral prefrontal cortex (DLPFC), a site within the frontal lobes highly associated with cognitive control [47] and emotional regulation [48]. Increased cerebral blood flow to this area may help strengthen neural circuitry in the frontal lobes, where cognitive and emotional control is located. This is consistent with the evidence linking executive functioning and low mood and suggests an indirect pathway whereby flavonoid consumption enhances cerebral blood flow, boosting executive functioning, and thus helping to inhibit cognitive features (i.e., rumination) that maintain depression.

An alternative explanation for the link between flavonoids and mood is the effect of anthocyanins (a subtype of flavonoids) on Monoamine Oxidase inhibition. MAO is involved in the oxidation of monoamines, some of which are neurotransmitters involved in the regulation of mood (e.g., serotonin, dopamine, and noradrenaline). MAO inhibitors have been used to treat mood disorders. Thus, the consumption of fruits high in flavonoids, such as blackcurrants, may significantly reduce MAO activity, thereby increasing circulating monoamines, and elevating mood [49]. This would suggest a direct pathway between flavonoid consumption and mood.

A third possible mechanism is the ability of flavonoids to mimic anxiolytic-like effects by binding to benzodiazepine receptors which influences the effects of Gamma Amino Butyric Acid (GABA) via $GABA_A$ receptors [50,51]. GABA is an inhibitory neurotransmitter present exclusively in the central nervous system. In addition to regulating cognition, decreased levels of GABA are associated with mood disorders. However, in the two studies reported here, the absence of an effect of flavonoid consumption on NA (a strong indicator of anxiety) suggests that the GABA hypothesis may not be a plausible explanation for the observed acute effects on PA.

6.5 Conclusion

This study demonstrated acute effects of blueberry flavonoid consumption on positive affect and no effect on negative affect in healthy children and young adults. Dietary interventions could play a key role in promoting positive mood and are a possible way to prevent dysphoria and depression. Given the potential implications of these findings for preventing depression, a disabling and common mental health problem in adolescents and adults, it is important to replicate the study and assess the potential to translate these findings to practical, cost-effective and acceptable interventions.

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Chapter 7. Addendum for Study 3: Effects of Acute Blueberry Flavonoids on Cognition in Young Adults and Further Mood Analysis

7.1 Introduction

As outlined previously, participants in this trial also completed an executive functioning task. A growing body of evidence suggests that dietary polyphenols from grapes, cocoa and blueberries are associated with cognitive benefits across all ages [1]. Acute and chronic flavonoid intervention studies have reported enhancement in cognitive functioning in animals and humans following supplementation [2-7]. The cognitive domains that have shown to improve due to flavonoid supplementation include working memory, episodic memory, psychomotor processing, and executive functioning [8-12]. It is hypothesised that flavonoid interventions affects different cognitive domains across the lifespan [2]. Improvement in executive functioning due to flavonoid intervention has been reported extensively in studies in young adults [2]. Additionally, a common symptom of depression is impaired cognitive functioning, with significant deficits in executive functioning [13]. Therefore, in addition to transient mood, adult participants' executive functioning was also assessed using Modified Attention Network Task. We hypothesise that, as the cognitive difficulty or demand of the tasks increases, participants will show an initial reduction in performance regardless of the treatment, flavonoid or matched placebo. However, after the flavonoid intervention, the performance would not decline as rapidly as when supplemented with placebo.

As a significant increase in positive affect after the wild blueberry was reported in study 3 [14], this data was further analysed by investigating the effects of the wild blueberry intervention on the subcategories of positive affect to explore what mood state was particularly influenced. The aim of this chapter, an addendum to the published paper, is to examine the data relating to the effect of flavonoids on executive function, as assessed by Modified Attention Network Task, and subcategories of positive affect, assessed by PANAS-Now.

7.2 Methods

7.2.1 Measures: In addition to the measures described in the previous study [14], cognitive performance was assessed using the Modified Attention Network Task (MANT) and was administered every time the PANAS was completed by participants. The Modified Attention Network Task assesses inhibitory control, selective attention, and response times (RTs; with RTs<100ms removed as these may have been intended for previous stimuli) [6]. Participants'

accuracy and reaction time were measured before and after drink consumption. As shown in Figure 1, arrow symbols, five in a row, facing either left or right ("<" and ">") were presented in white against a black background. The centre arrow was either congruent (i.e. <<<<< or >>>>>) as in Figure 1A or incongruent (i.e. <<><< or >><>>) with pair of arrows on either side as Figure 1C. These stimuli were presented in two difficulty settings, medium load, where only one row of arrows was presented (congruent, 1A or incongruent 1C) and high load, where two rows of arrows were displayed (congruent Figure 1B and incongruent Figure 1D). Participants were presented with one hundred trials with the direction of the arrows, congruence, and load, randomised and appearing with equal probability. Pseudorandom stimulus intervals of 1000, 1300 or 1500ms was presented following the stimulus, which was displayed for 120ms. Participants were instructed to use the left and right keys on the keyboard to indicate the direction of the presented stimuli (i.e. the centre arrow). Accuracy and response times for both congruent and incongruent trails were measured separately.

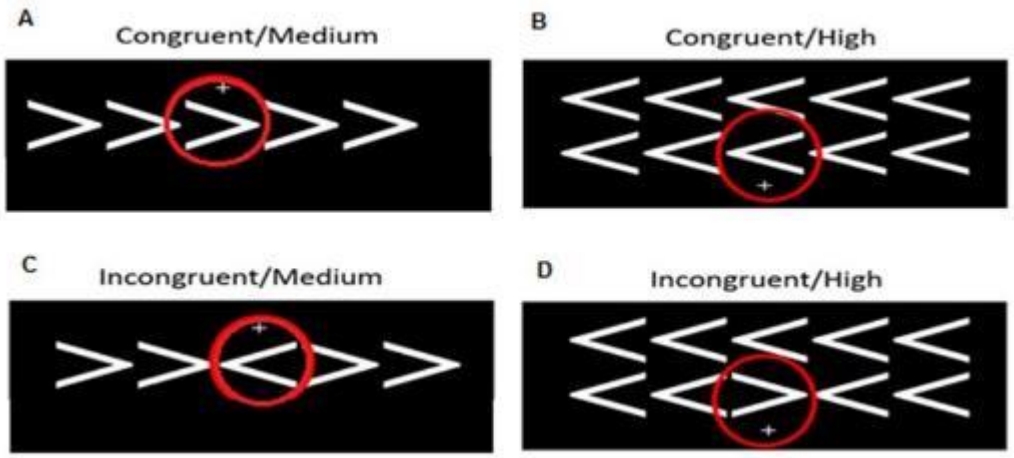

Figure 1. (A) Shows congruent stimuli at medium difficulty load. (B) Shows congruent stimuli at high difficulty load. (C) Shows incongruent stimuli at medium difficulty load. (D) Shows incongruent stimuli at high difficulty load.

Effects of flavonoids on positive affect was further explored. Positive affect was measured using the Positive and Negative Affect Scale (PANAS-NOW), where participants rated ten positive mood items including Interest, Alertness Attentiveness, Excitedness, Enthusiasm, Inspiration, Pride, Determination, Strength and Activeness. These scores were added to determine an overall positive affect score. For further analysis, the positive mood items were grouped together to construct four subcategories; 'Attentive' (items include interests, alertness and attentive) 'Excited' (items included excitedness, enthusiastic and inspired),

‘Proud’ (items include pride and determined) and ‘Strong’ (items includes strong and activeness). The scores for each item in the subcategory was added to determine the score of the subsequent category.

7.2.2 Analysis: All analysis was performed using SPSS (version 22.0). Cognitive functioning was assessed by accuracy and reaction time on the Modified Attention Network Task. A four-way, drink (2) x load (2) x session (2) x congruence (2) repeated measures ANOVA (analysis of variance) was performed. Any significant main effects of drink, session, congruence and load, and interaction effects of drink x session x congruence x load were explored using Bonferroni corrected post hoc t-tests.

Positive affect subcategories, Attentive, Excited, Proud and Strong were dependent variables in a two-way repeated measures ANOVA, with drink and session as independent variables Significant main effects and interactions were again explored with Bonferroni corrected post-hoc t-tests.

Baseline differences in cognitive functioning was examined using one-way ANOVA with Drink (placebo and WBB) as the independent variable.

Association between cognitive functioning and positive affect scores reported in the previous chapter was assessed using Multiple Linear Regression with the accuracy and reaction times when presented with stimuli of different cognitive difficulty, as the predictor variable (eight in total) and positive affect score as the criterion variable.

7.3 Results

7.3.1 Executive functioning: In terms of executive function, there was no significant differences at baseline. Interaction effects of drink x congruency ($F(1,20)=0.856$ $p=0.366$), drink x load ($F(1,20)=1.621$ $p=0.218$), congruence x load ($F(1,20)=0.579$ $p=0.218$), drink x session ($F(1,20)=0.003$ $p=0.961$, congruence x session ($F(1,20)=0.101$ $p=0.754$), load x session ($F(1,20)=0.0095$ $p=0.761$), drink x congruency x load ($F(1,20)=1.007$ $p=0.327$), congruency x load x session ($F(1,20)=0.007$ $p=0.934$), drink x congruency x load ($F(1,20)=2.409$ $p=0.136$) and drink x session x congruency x load ($F(1,20)=0.927$ $p=0.347$) on accuracy. There was a significant interaction effect between type of drink, load of the task and session on accuracy, $F(1,20)= 5.60$ $p=0.028$. Participants were more accurate on high difficulty load trials after the consumption of blueberry drink ($M=0.94$ $SE=0.019$) as compared to the placebo drink ($M= 0.925$, $SE= 0.025$). However, post hoc analysis revealed no significant differences in the mean accuracy scores before and after the consumption of

both the drinks for trials of both levels of difficulty (Figure 2), showing no wild blueberry produced benefits as the cognitive demand increases. Similarly, there was no significant main effect of drink ($F(1,20)=2.731$ $p=0.114$), Load ($F(1,20)=0.015$ $p=0.903$), and session ($F(1,20)=0.035$ $p=0.835$) on MANT accuracy. However, there was a significant main effect of congruency on participants' accuracy, $F(1,20)=8.25$ $p=0.009$. Further post hoc analysis showed that there was a statistically significant difference between the mean accuracy score when the congruent task (M=0.99, SD=0.002) and incongruent task (M=0.88, SD=0.015) was performed p=0.009. As expected, participants performed more accurately on the congruent trails than on to the more cognitively demanding incongruent trials.

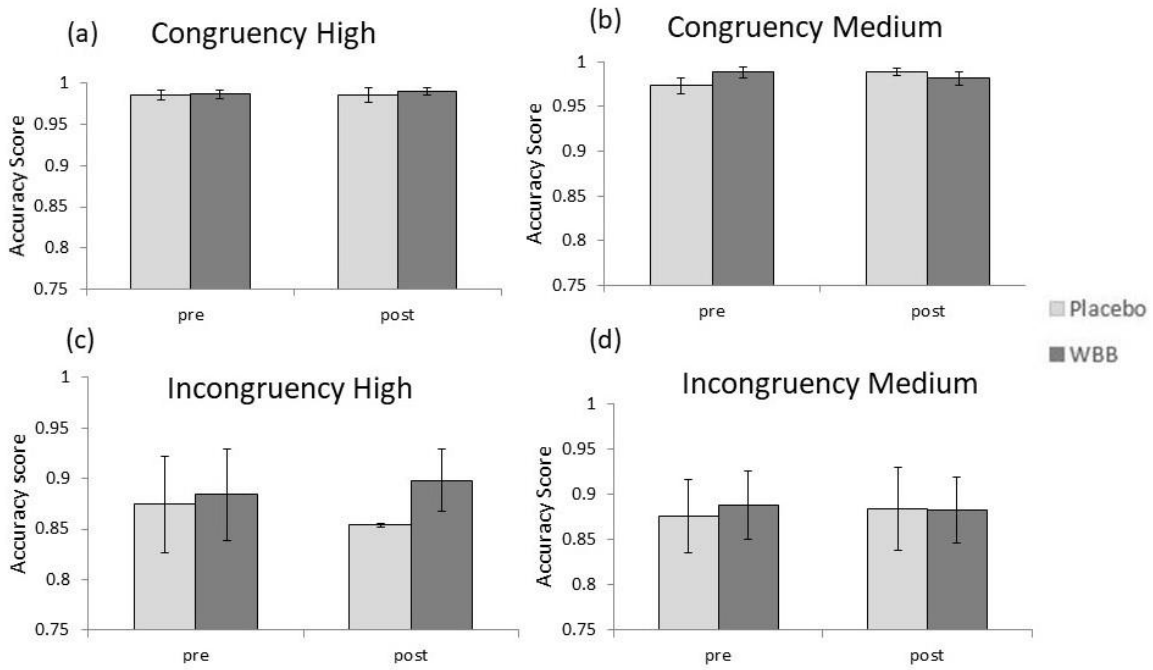

Figure 2. Mean Accuracy score on MANT in young adults aged 18-21 years: (a) Mean Accuracy score when congruent/high stimuli was presented, pre and post-consumption of placebo and intervention drinks. (b) Mean Accuracy score when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks. (c) Mean Accuracy score when incongruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks. (d) Mean Accuracy score when incongruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks.

Similarly, as shown in Figure 3, there was no significant main effect of drink, $F(1,20)= 0.290$ $p=0.596$ or session $F(1,20)= 0.006$ $p=0.94$ on participants' reaction time. The only significant main effect for reaction time was of congruency $F(1,20)=136.65$ $p<0.001$ and load $F(1,20)=35.5$ $p<0.001$. As expected, participants reacted significantly faster during congruent trials (M=406.6 SE=11.27) than on incongruent trials (M=465.99 SE=12.49) and took more time to react when the load difficulty of the trials were higher (M=441.7 SE=12.04) compared to medium difficulty trials (M=430.85 SE=11.27). There was no intervention related

statistically significant interaction effect observed on reaction time. Drink by congruency; F(1,20)=0.112 p=0.741, drink by load F(1,20)=1.767 p=0.199, congruency by load F(1,20)=0.454 p=0.508, load by session F(1,20)=1.902 p=0.183, drink by session F(1,20)=0.629 p=0.437, congruency by session F(1,20)=1.525 p=0.23, drink by congruency by load F(1,20)=0.55 p=0.465, drink by load by session F(1,20)=0.528 p=0.476, congruency by load by session F(1,20)=0.473 p=0.50, drink by congruency by load by session F(1,20)= 0.003 p=0.959. However, there was a trend towards significance for the interaction of session, type of drink and congruency (F(1,20)=3.653 p = 0.07) on reaction time.

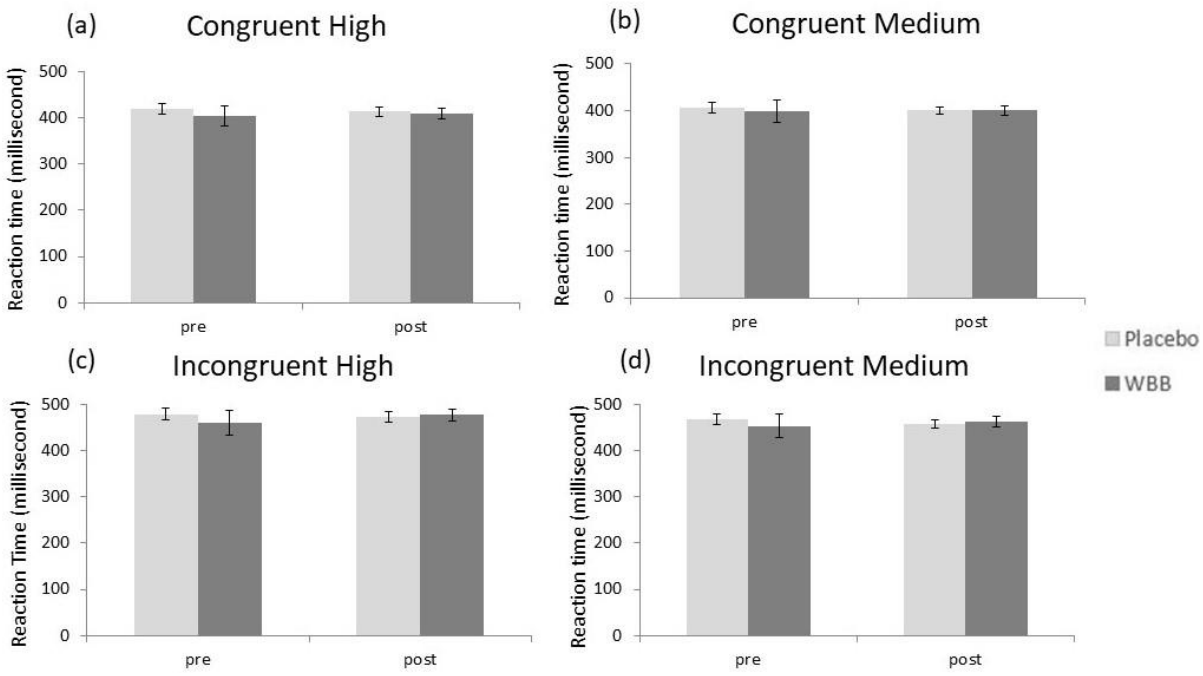

Figure 3. Mean Reaction time on MANT in young adults aged 18-21 years : (a) Mean reaction time when congruent/high stimuli was presented, pre and post-consumption of placebo and intervention drinks. (b) Mean reaction time when congruent/medium stimuli was presented, pre and post-consumption of placebo and intervention drinks. (c) Mean reaction time when incongruent/high stimuli was presented pre and post-consumption of placebo and intervention drinks. (d) Mean reaction time when incongruent/medium stimuli was presented pre and post-consumption of placebo and intervention drinks

7.3.2 Subcategories of Positive Affect: Upon further analysis of the sub-categories of Positive Affect, there was no main effect of drink on any of the subcategories of positive affect. There was, however, a significant main effect of session on the subcategories Attentive (F(1,20)=14.613 p=0.001) and Excited (F(1,20)=10.516 p=0.004), (Figure 4 a & b respectively). Participants reported feeling more attentive (pre: M=7.619 SD=0.427, post: M=9.262 SD=0.391) and more excited (pre: M=6.31 SD=0.455, post: M=7.833 SD=0.497) after the consumption of drinks. There was a significant interaction effect between type of

drink and session for the subcategories Excited (F (1,20) =10.909 p=0.004) and Proud (F(1,20)=5.656 p=0.027). Post hoc analyses showed that students reported feeling significantly more excited after the consumption of blueberry drink (M=8.62 SD=3.15) as compared to before consumption (M=6.10 SD=2.86), p=0.003.) This effect was, however, not significant after the consumption of placebo drink (pre: M=6.52 SD=3.09, post: M=7.05 SD=3.17, Figure 4b). Increase in the feeling of pride was reported after the consumption of drinks with students feeling slightly more proud after the consumption blueberry drink (pre: M=4.43 SD=2.18, post: M=5.48 SD=2.14) as compared to placebo drink (pre: M=4.62 SD=2.13 post: M=4.48 SD=1.75), however, this effect was not significant p=0.074 (Figure 4c). The interaction effect between drink and session was trending towards being significant for the subcategory Strong F (1,20) = 4.005 p=0.059 (Figure 4d) where again participants reported increased feelings of 'Strong' after the blueberry intervention.

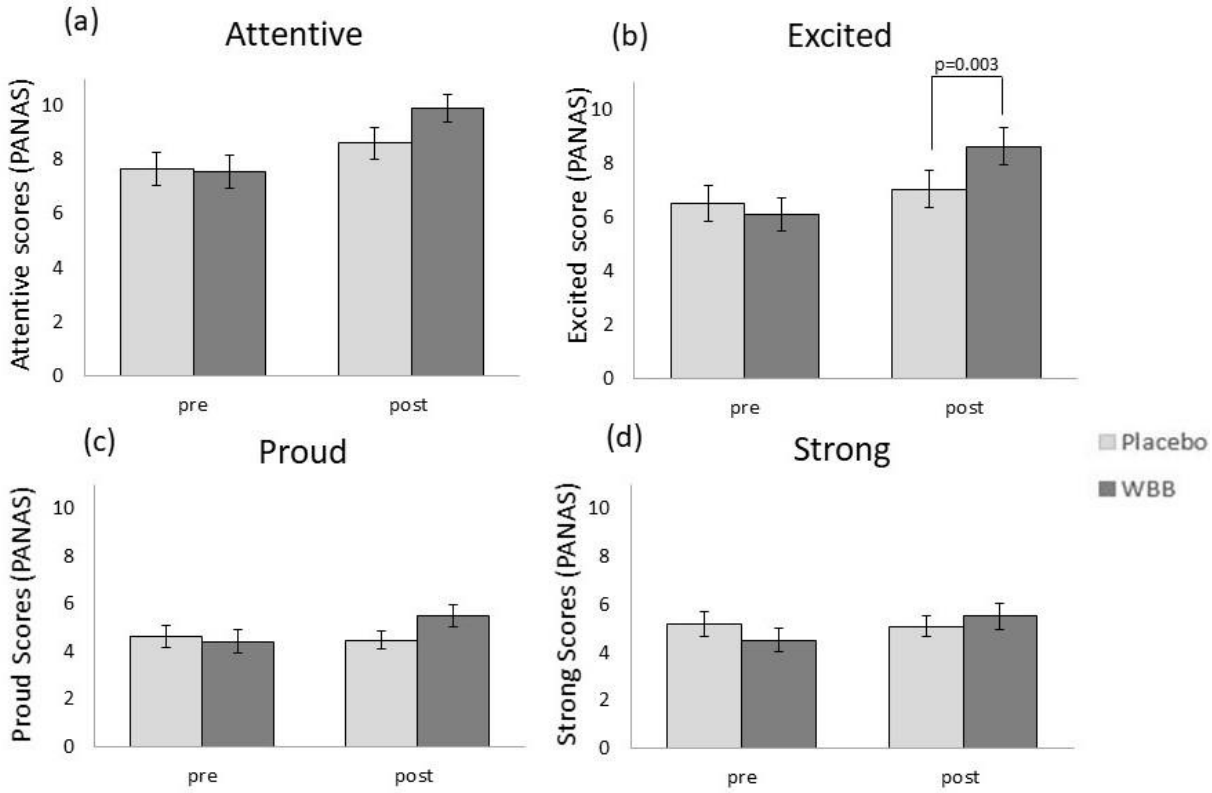

Figure 4. Mean PA sub-categories scores in young adults aged 18-21 years: (a) Mean Attention scores 2 hours post-consumption of placebo and intervention drinks. (b) Mean Excited scores 2 hours post-consumption of placebo and intervention drinks. (c) Mean Proud scores 2 hours post-consumption of placebo and intervention drinks. (d) Mean Strong scores 2 hours post-consumption of placebo and intervention drinks.

7.3.3 Association between Cognition and Positive Affect: Multiple linear regression was carried out to determine the association between cognitive functioning (accuracy and reaction time) and positive affect. Positive affect scores were not significantly correlated to

participants accuracy or reaction time when presented with congruent and incongruent stimuli at both medium and high cognitive difficulty load. The multiple regression model, with all eight predictors, was not significant, $R2=0.286$, $F(8,20)=0.6$, $p=0.76$, indicating that cognitive performance did not predict participants' positive affect scores.

7.4 Discussion

This addendum to the randomised, placebo-controlled, double-blind study investigating the effects of acute consumption of a flavonoid-rich wild blueberry drink on transient mood of healthy young adults, also explored its effects on cognition and carried out further analysis of positive affect that may have been specifically influenced by the intervention. As expected, response interference was evident, though not significant, with lower accuracy and slower response time on incongruent higher load trials. Participants were more accurate and faster (lower reaction time) on congruent trials when compared to incongruent trials. Though not significant, participants were more accurate on higher load trials after the consumption of the wild blueberry intervention when compared to the matched placebo. No other significant effects of the intervention were observed on participants' accuracy or reaction time (cognition). Previous studies found that wild blueberry consumption resulted in a significantly quicker reaction time [(15)] and overcame the effects of incongruent high difficulty load trials, the most cognitively demanding trials [(6)]. These effects were not observed in this study. The previous studies were conducted in the afternoon with participants given low flavonoid lunch before the test session, controlling for the effects of sugar on cognition. This study was conducted in the mornings, with participants only instructed to follow a low flavonoid diet 24 hours before the research assessment. Therefore, we could not control the type of breakfast the participants had consumed, if any. Therefore, any effect on cognition due to flavonoids may have been masked by the sugar content of the intervention and placebo drinks. The difference in age of the participants recruited in this study and the previous study may also be an additional factor contributing to the lack of effects observed of flavonoids on cognition. The Modified Attention Network Task may not have been cognitively challenging enough for young adults as it had been in children. Further studies should consider including tasks that are more cognitively challenging for this particular age group and, those that measure a wider range of cognitive abilities, including working memory and verbal fluency. Since no significant blueberry related benefits were observed on cognition and no significant association between cognition and positive affect was demonstrated, it may also be that the effects that were observed on PA, due to blueberry consumption, were not via improvement in

cognition, and that there potentially may be other underlying mechanisms, mentioned in the previous chapter, accounting for this improvement in mood.

Though overall there was an increase in subcategories, Attentive, Proud and Strong, the change was not significant. There, however, was a significant increase in ratings of Excited after the consumption of wild blueberry drinks. Moods are complex processes that involve several response channels, including cognition and neural activity, resulting in changes in autonomic and neuroendocrine systems. However, the ability of flavonoids and their metabolites to cross the blood brain barrier may play a role in the elevated feeling of these particular mood states [16]. Flavonoids vasodilation properties are not restricted to peripheral systems but also been observed to result in increased cerebral blood flow [4,5,8]. As mentioned in the previous study [14], another possible mechanism by which mood effects are being mediated is via inhibition of monoamine oxidase benefiting monoaminergic neurotransmission [10]. However, the exact mechanism by which wild blueberry flavonoids affect the feelings of excitement, enthusiasm and inspiration is unclear.

7.5 Conclusion

In conclusion, this addendum shows the specific mood states within the positive affect that were significantly improved due to acute blueberry flavonoid intervention. Though the exact mechanisms by which these improvements are brought about are still unknown, this analysis may aid future studies in respect to identifying specific mood pathways to explore, such as Gamma Amino Butyric Acid (GABA) pathway and oxidation of monoamines. Additionally, it is also clear that further investigation is required to observe the beneficial effects of flavonoids on cognition and mood in young adults**.**

7.6 References

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Chapter 8: Study 4: Effects of Acute Blueberry Flavonoids on Mood and Cognition in Healthy and Young Adults with Depressive Symptoms

Abstract

Consumption of flavonoids is associated with improved transient mood and cognitive functions. In particular, wild blueberry flavonoids have shown to increase positive affect in 7-10-year-old children and in 18-25-year-old young adults, 2 hours post consumption. Similarly, an improvement in executive function and short-term memory was observed, in children aged 7-10 years, 2-6-hour post wild blueberry intervention. As participants' depressive symptoms were not measured upon recruitment, it is unclear if the beneficial effect of flavonoid on transient mood were observed in healthy participants or also those with low mood. The aim of this study was to explore the effects of blueberry flavonoids on transient mood and cognition in both young adults with and without elevated symptoms of depression. Therefore, in this double blind, placebo controlled, crossover study, we screened young adult participants for symptoms of depression prior to recruitment. A total of 33 university students (29 females) aged 18-25 years were recruited. Twelve participants reported elevated symptoms of depression on the Patient Health Questionnaire (PHQ-9), based on a standardized clinical cut off. Participants consumed 250ml of wild blueberry drink (253mg anthocyanins) and a matched placebo in a randomised order with a minimum of three day wash out period. Their transient mood (Positive and Negative Affect Schedule; PANAS), and measures of selective attention, verbal fluency, and working memory were assessed before and 2 hours after they consumed both drinks. There was no significant difference in positive or negative affect or on any of the cognitive measures after consuming the experimental and control drinks, in either the healthy participants or those with elevated symptoms of depression. This study requires replication due to the small sample size of those with depression, as any beneficial effects of flavonoids on this group may not have been observed due to low statistical power.

8.1 Introduction

Dietary flavonoids, representing a diverse range of polyphenols, are shown to have beneficial effects on health. They are present in substantial amounts in commonly consumed fruit, vegetables, grains, herbs, and beverages. Their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties are well documented. Evidence suggests that flavonoid intervention improves coronary and vascular functioning [1,2], in addition to enhancing cognitive functions [3-5]. Acute intervention of flavonoids has shown improvements in psychomotor processing speed [6,7], episodic memory [8-10], working memory [11,12], attention [11,13] and executive function [6,13]. Some acute effects of flavonoid consumption also include an increase in cerebral blood flow which is proposed to protect against neuronal stress and positively mediating signalling pathways in the brain [14-16]. There is increased evidence that there may also be a relationship between flavonoid consumption and mental health, with for example, epidemiological evidence of an association between lifetime flavonoid consumption and reduced risk of depression in older women [17]. A recent systematic review of epidemiological research found cross-sectional and longitudinal associations between consumption of a 'healthy' diet (i.e. consumption of fruit and vegetables) and better mental health in children and young adults [18]. A large well-controlled 10 year follow up, epidemiological study (82,643 women), examining associations between habitual intakes of dietary flavonoids and depression risk showed that individuals consuming diets higher in flavonoids presented a lower depression risk, particularly amongst older women [17]. A similar study assessed symptoms of depression and the total habitual intake of polyphenols over a year in 1572 adults and found that higher dietary intake of flavonoids was inversely associated with depressive symptoms [19]. However, experimental studies directly investigating the causal relationship between flavonoid consumption and depressive symptoms are scarce and conflicting. Acute wild blueberry supplementation has been shown to enhance positive transient mood in healthy children and young adults [20]. Nevertheless, numerous double-blind, crossover studies using a variety of dietary flavonoids, however, have not found any acute effects of wild blueberry [21], blackberry [22], grape juice [23], chocolate drink [24] and green tea [25] flavonoids on mood in healthy adults.

The aetiology of depression is complex with many interacting genetic, social, psychological and physiological risk factors. For example, neuronal stress and disruption in signalling pathways are suggested to contribute to the development of depression [26]. Furthermore, mood regulation involves a wide range of cognitive processes such as directing attention and thoughts (away from negative stimuli), mobilizing problem solving, and planning, initiating and directing behaviours. Cognitive impairment is a common symptom of depression [27,28].

This impairment has also been identified across several cognitive domains such as executive functioning [29-34] episodic memory [35-38], semantic memory [39-42], visuo-spatial memory [43,44], and information processing speed [45-50]. Though not regularly replicated, the levels and patterns of impairment differ in patients, with increased severity in depression relating to increased impairment in overall cognitive ability [27,51-53].

Cognitive symptoms due to depression are thought to be a result of dysregulation of interacting neural networks involving prefrontal cortex, hippocampus, amygdala, basal ganglia, and anterior cingulate [54]. Interactions between cognition and emotion have been linked with interactions between hippocampus and prefrontal cortex. Also, decreased hippocampal activity is thought to be related to increased negative bias and to the inability of the amygdala to inhibit information [55]. Additionally, monoamine networks that involve serotonergic, noradrenergic and dopaminergic pathways are involved not only in mood regulation but also contribute to an array of cognitive functions. Evidence suggests that low levels of serotonin not only have an impact on mood but also have a negative effect on cognitive functions [56].

Examining the relationship between dietary flavonoids and depression symptoms, including cognitive functioning is complicated by the different ways in which 'mood' and 'depression' are defined and assessed. "Mood" is usually conceptualised as a transient state, linked with a clear external trigger [57,58]. However, 'mood' is also used to refer to a more persistent disruption in affect, i.e. low mood, which is more synonymous with a core symptom of major depressive disorder. Measures used to assess 'mood' therefore either, target a specific transient affective state (i.e. positive affect or negative affect), or a broad dimension of emotion, i.e. 'low mood', that is theorized to underline global domain. Tools that are used to measure current transient mood, as have been used in previous studies, differ therefore from those that aim to assess sustained low mood in the context of depression. Therefore, any inferences made about sustained low mood or depression using transient mood measures is inappropriate. Using a validated screening tool for depression that assesses the affective and cognitive components of depression, as used in this study, is required to draw more accurate inferences about the relationship between dietary flavonoids and symptoms of depression.

Another reason for inconsistency in the literature may be that different cognitive domains may be sensitive to the flavonoids at different ages, reflecting lifespan changes as reported in a recent review [59]. There may be sensitive periods of development e.g. adolescence and young adulthood when flavonoids are more likely to have an impact on the neural circuitry relating to emotions.

The specific effects of acute wild blueberry flavonoid on transient mood and cognition in young adults with elevated symptoms of depression has not yet been investigated. This double-blind placebo-controlled study, therefore, aims to investigate the effects of wild blueberry flavonoids on cognition and transient mood in a group of healthy adults and those with elevated symptoms of depression, two hours post intervention.

8.2 Methods

8.2.1 Ethics: This research was reviewed and given a favourable ethical opinion for conduct by the University of Reading Research Ethics Committee

8.2.2 Participants: Undergraduate students, aged 18-25 years, were recruited from the University of Reading. Based on the effect size from the pilot study [20] a power calculation (using G power) showed that in a cross over design, 21 students would give 71% power. Thus, to achieve power of 95%, 40 students needed to be recruited, 20 healthy participants and 20 participants with elevated depression symptoms. Forty participants were recruited of whom 7 participants dropped out after the practice session and were excluded. This resulted in 33 participants that were included in the study. All participants provided written consent and were screened for food related allergies and other health conditions, e.g. diabetes, heart disease, blood pressure, thyroid, kidney, and liver diseases that would exclude them from the study. Participants were administered the intervention and placebo treatments in a randomised order, generated using excel. The experimental and placebo drinks were coded A and B and the sequence in which they were given to each participant was saved in a password protected spreadsheet. Both the researchers and the participants were blind to the order in which the drinks were allocated and consumed.

8.2.3 Interventions: Both interventions (wild blueberry and placebo) were measured and packaged into silver opaque sachets at the University of Reading. Sachets were identical for the wild blueberry and the placebo drink, and neither the researchers nor the participants knew what their sachets contained. The packets of wild blueberry contained 30g of freeze-dried wild blueberry powder (containing ~253mg anthocyanins). Placebo packets were matched to the wild blueberry drink for sugars (7.99g glucose and 8.9g fructose) and vitamin C (4 mg). Analysis of anthocyanin content was carried out by independent researchers from the University of Reading using the methods described in Rodriguez-Mateos et al ., 2012 [60] indicating anthocyanin content of 8.43 mg/g which, given a freeze dried to fresh ratio of 7/1, is equivalent to 120.5 mg/100g fresh (see Table 1). All drinks were prepared onsite, by a graduate assistant independent of the study, no more than 20 minutes prior to consumption.

Drinks were prepared by adding 30ml of Rocks Orange Squash (containing 13.2mg total polyphenols Narirutin & Hesperidin) and 220ml of water to the contents of the sachets in an opaque cup to ensure that both the researcher and the participant remained blind to the treatment.

Table 1. Showing the anthocyanin content of the intervention drink.

	mg/g freeze dried	Stdev	mg/ 100 g fresh BB	Stdev
Delphinidin	3.29	0.21	46.94	3.03
Cyanidin	1.17	0.07	16.64	0.95
Petunidin	1.58	0.08	22.50	1.16
Peonidin	0.37	0.02	5.24	0.30
Malvidin	2.04	0.10	29.14	1.50
Total	**8.43**	**0.48**	**120.47**	**6.92**

8.2.4 Measures: Patient Health Questionnaire-9 [(61)] (Appendix 6); Depression symptoms were assessed using Patient Health Questionnaire-9 (PHQ-9). The PHQ-9 has good test-retest reliability and internal consistency [(61)]. It contains 10 questions, 9 items assessing the symptoms included in the DSM-IV criteria for major depressive disorder, as well as an additional item assessing psychosocial impairment. Participants were asked to rate each item in relation to their symptoms in the past 2 weeks on a 3-point Likert scale (not at all = 0, several days= 1, more than half the days = 2, nearly every day = 3). Total scores on the PHQ-9 ranged from 0 to 27 and the recommended clinical cut off score ranges are 0-4 (no depression), 5-9 (mild depression), 10-14 (moderate depression), 15-19 (moderately severe depression), and 20-27 (severe depression). Participants who scored 4 or below made up the healthy group and those who scored 5 or above on the PHQ-9 were allocated to the 'at risk' group.

Positive and Negative Affect Schedule-NOW [(62,63)] (Appendix 5): Current mood (i.e. transient affect) was assessed using the Positive and Negative Affect Schedule-NOW (PANAS-NOW) before each of the test sessions and 2 hours after participants consumed the drinks. The PANAS is a valid and reliable 20 item self-report measure of positive affect (PA – 10 items) and negative affect (NA - 10 items) that can be used on multiple test occasions. Participants rated the degree to which they were currently experiencing each item on a 5-point Likert scale ranging from 'very slightly' to 'extremely'. Ratings of positive and negative items were summed to calculate an overall positive affect and overall negative affect score.

Modified Attention Network Task (MANT [64]): This included a cue target and flanker task, and was used to measure response times (RTs; with RTs< 100ms removed) and selective

attention, under different levels of cognitive demand. In accordance with Whyte et al. [64], stimuli load, duration and cueing were manipulated to modify the cognitive demands. Five arrow symbols "<" and ">" were presented in white against a black background. The centre arrow was either congruent (i.e. <<<<< or >>>>>) or incongruent (i.e. <<><< or >><>>) with pair of arrows on either side. One hundred trials were presented, with the direction of the arrow and congruence randomised and appearing with equal probability. The stimulus was displayed for 120ms and then followed by a pseudo-random stimulus interval of 1000, 1300 or 1500ms. Participants were instructed to use the left and right keys on the keyboard to indicate the direction of the presented stimuli (i.e. the centre arrow). Accuracy and response times for both congruent and incongruent trails were measured separately.

The Controlled Oral Word Association Task (COWAT) [65]: to assess verbal fluency participants were instructed to name as many words as possible that begin with a specified letter within one minute. They were told that names of places and people and compound words would not count. For example, if they were asked to list words beginning with the letter "L", proper nouns, e.g. 'London' and 'Lucy' would not be accepted. If the word "lose" was used, then they could not also use "losing", 'loser' and 'lost'. Participants were presented with 3 letters, one at a time, and asked to generate as many words beginning with that letter, within a minute. The letters used were randomly selected from C, F, L, P, R, W, A, S [64] and presented on a computer screen and the response recorded. The mean number of words generated across the 3 letters was calculated.

The Keep Track Task [66,67]: KTT was used to assess working memory. This assesses how participants update and modify information in working memory. They were presented with a set of target categories (animals, colour, furniture, relatives, and fruits) with seven words in each category. Three to five target categories were presented at the beginning of the trial. Words from each category were presented sequentially, 2-3 words from each category. Participants were required to remember and write down the most recent word that had been presented from each of the target category at the end of each trial. This task had total of nine trials. The proportion of correctly identified words was calculated.

Computerized Serial 3s subtraction tasks [68]: This was used as a measure of working memory and concentration. A randomly generated number between 800 and 999 was displayed on the computer screen and participants were instructed to subtract three from this number, in a serial fashion, as quickly and accurately as possible. The numeric keypad on the keyboard was used to record the responses. Each digit entry was displayed in an asterisk on the screen. Pressing the enter key on the keyboard indicated the end of each response and the next three-

digit response was entered. In case of an incorrect response, the subsequent response was scored positively if the response was correct in relation to the new number. The task was scored for number of correct responses and lasted for two minutes. Computerized serial 7s task was identical to serial 3s, except participants were required to subtract seven from the number presented.

8.2.5 Statistical Analysis: All statistical analysis was conducted using SPSS version 22. The relationship between age and depressive symptoms was assessed using Pearson's correlation. Mixed Analysis of Covariance (ANOVA) was used to explore the effects of intervention in the healthy and 'at risk' groups in addition to exploring the between group effects of the intervention. The acute effects of wild blueberry flavonoids on transient affect, verbal fluency (COWAT) and working memory (KTT, Serial 3's and 7's) was analysed using three way mixed ANOVA with drink (placebo, wild blueberry) and session (pre and post intervention) as within group independent variable and mood state (healthy or at risk) as between group independent variable. Positive affect scores, negative affect scores, COWAT scores, KTT, Serial 3's and 7's scores were the dependent variables. Accuracy and reaction time on the Modified Attention Network Task were analysed using a five way mixed ANOVA, with the within subjects factor of drink (2) x session (2), congruency (2) x load (2) and the between subjects factor of mood state (2) as mentioned above. Bonferroni corrected t-tests were used to investigate all significant fixed effects and interactions. To assess any differences between groups at baseline one-way ANOVAs were performed on baseline mood and cognition data. The correlation between depressive symptoms and cognition at baseline was assessed using multiple linear regression with PHQ-9 scores as the criterion variable and cognitive measures as predictor variables.

8.3 Results

Thirty-three participants were recruited, (29 females, 4 males) aged 18-25 years (Mean 20.8, SD1.7). Twenty-one participants were allocated in the healthy group, as their scores on the PHQ-9 were below 4 (Mean 1.29, SD = 1.31). Twelve students reported elevated symptoms of depression with scores above 4 on the PHQ-9 (Mean 8.83, SD = 2.29). The number of males and females with elevated symptoms of depression is reported in the frequency Table 2 below. None of the participants were diagnosed with any mental health conditions nor were ever on any treatment/medication for these conditions. There was no significant correlation between age and PHQ-9 ($r=-0.1$, $N=33$, $p=0.5$). No significant differences at baseline in mood or any of the cognitive measure was revealed between the two groups ($p>0.05$).

There were no significant differences between the two groups in mean levels of positive affect, negative affect, or overall levels of any of the cognitive tasks. However, there was a significant difference in the baseline accuracy on more demanding versions of the tasks. On the flanker task, those in the healthy group were more accurate than those in the low mood group (p=0.017). On the more demanding serial 7s and KTT tasks, those in the healthy group preformed significantly better than participants with low mood (serial 7s: p=0.008, KTT: p= 0.006).

Table 2: showing the number of male and females with and without symptoms of depression.

	Healthy (PHQ-9 score < 4)	**Elevated Symptoms (PHQ-9 score >5)**	**Total**
Male	3	1	4
Female	18	11	29
Total	21	12	33

Figure 1 shows mean Positive Affect before and after consumption of the placebo and flavonoid drinks in the healthy and 'at risk' groups. There was no significant main effect of Drink (F (1,28)=0.386 p=0.539) or Session (F(1,28)=0.07 p=0.79) on positive affect. There was also no significant interaction effect of drink by mood state (F (1,28)=0.61 p=0.97), session by mood state (F(1,28)= 0.001 p=0.97) or drink by session (F (1,28)= 2.75 p=0.11) on positive affect. There was a significant interaction effect of drink by session by mood state (F(1,28)=4.42 p=0.045). Further analysis revealed no significant change in positive affect in healthy participants after consuming both the placebo (pre: M(SD)= 27.58(7.71), post: M(SD)= 27.85(7.09)) and the blueberry drink (pre: M(SD)= 26.14(6.19), post: M(SD)= 26.86(6.30)). Similarly, no significant change in positive affect was observed in participants with elevated symptoms of depression. Contrary to the hypothesis, these young adults did however report an increase in positive affect after consumption of the placebo drink (pre: M(SD)= 26.64(6.67), post: M(SD)=29(5.11)) and a decrease in positive affect after the consumption of wild blueberry drink (pre: M(SD)=27.6(6.04), post: M(SD)=25.45(8.82) as shown in Figure 1(a).

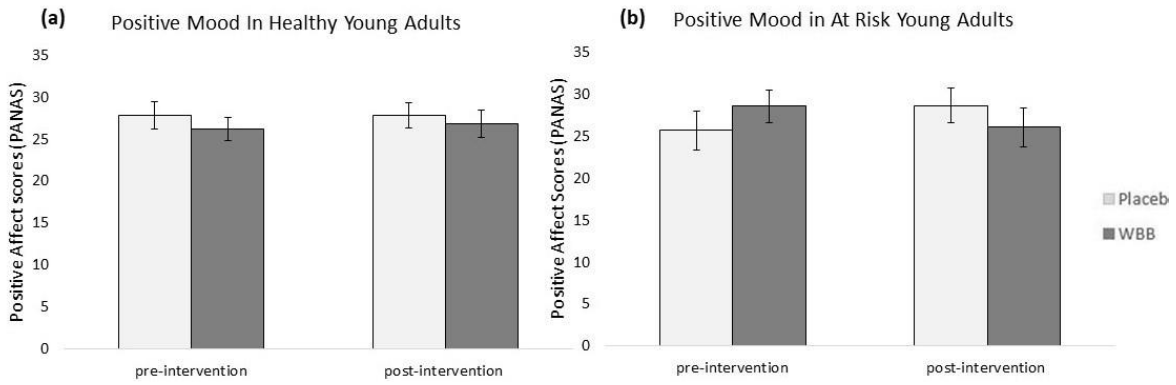

Figure 1. Mean (± standard error of the mean) PANAS-NOW, Positive affect Mood scores in adults aged 18–25 years: (a) Mean PA scores pre- and 2 hour post-consumption of placebo and intervention drinks in Healthy individuals ; (b) Mean PA scores pre- and post-consumption of placebo and intervention drinks in individuals with elevated symptoms of depression (At risk of depression).

Mixed ANOVA revealed no significant main effect of drink on negative affect (F(1,28)= 3.40 p=0.076). However, there was a significant main effect of session on negative affect (F(1,28)= 4.90 p=0.035) where there was a decrease in participants' self-reported negative affect after both the intervention and placebo drink. This change was observed in both the healthy (placebo: pre M(SD)=12.25(3.00), post M(SD)=11.95(3.10); Blueberry: pre M(SD)= 12.05(2.67), post M(SD)=11.50(2.16)) and at risk groups (placebo: pre M(SD)=15.40(5.19), post M(SD)=14.00(5.66); Blueberry: pre M(SD)=13.80(2.97), post M(SD)=12.2(1.75)). There was no significant interaction of drink by mood state (F(1,28)=1.53 p=0.23), session by mood state (F(1,28)=1.53 p=0.23), drink by session (F(1,28)=0.23 p=0.64) or drink by session by mood state (F(1,28)=0.003 p=0.96). Figure 2 shows the negative affect scores before and after consumption of the placebo and flavonoid drinks in both healthy (2a) and at risk (2b) group.

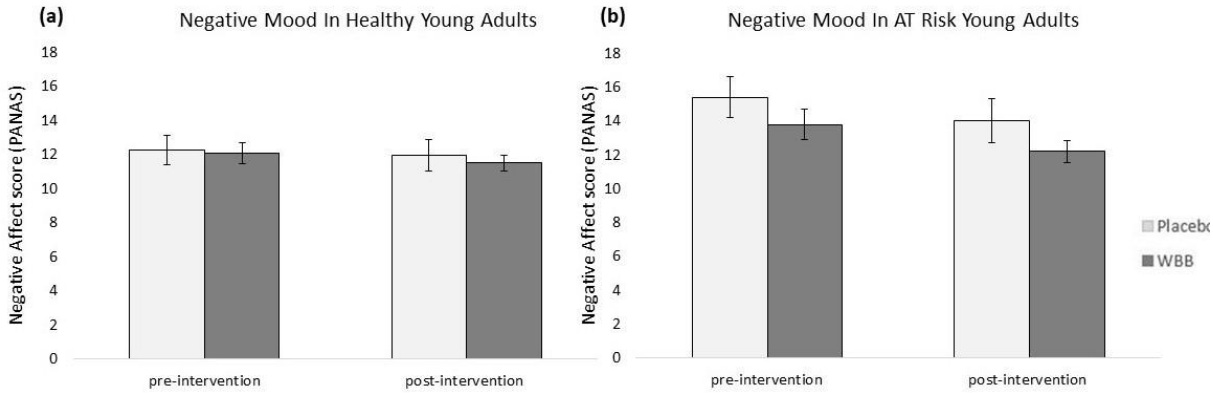

Figure 2 Mean (± standard error of the mean), Negative affect Mood scores in adults aged 18–25 years: (a) Mean NA scores pre- and 2 hour post-consumption of placebo and intervention drinks in Healthy individuals ; (b) Mean NA scores pre- and post-consumption of placebo and intervention drinks in individuals with elevated symptoms of depression (At risk of depression).

Analysis of the Modified Attention Network Task data revealed that response interference was evident with participants showing significantly lower accuracy, $F(1,28)=47.12$ $p<0.001$ and slower response time, $F(1,28)=364.84$ $p<0.001$, for incongruent trials compared to congruent trials. There was a significant effect of congruence in both the healthy and at risk group, $F(1,28)=7.13$ $p=0.0120$. Data were analysed separately for congruent and incongruent trials. There was no significant effect of session, $F(1,28)=0.045$ $p=0.83$, drink, $F(1,28)=0.89$ $p=0.35$ or load, $F(1,28)=1.38$ $p=0.25$, on accuracy scores. There was a significant interaction of load by congruency between the two groups, $F(1,28)= 4.49$ $p=0.043$ and drink by load by congruency, $F(1,28)=4.81$ $p=0.037$. Participants with elevated depressive symptoms were less accurate in all the trials, compared to healthy participants. Figures reporting the mean accuracy scores of both groups is shown in Appendix 7.

Similarly, there was no significant main effect of drink, $F(1,28)=0.66$ $p=0.42$, or session, $F(1,28)=1.85$ $p=0.184$ on participants' reaction time. There was a significant main effect of load, $F(1,28)=35.82$ $p<0.001$ on reaction time; participants were slower in responding when high load trials were presented. There was a significant interaction effect of drink by congruency, $F(1,28)=71.66$ $p<0.001$, session by congruency, $F(1,28)=149.87$ $p<0.001$, load by congruency, $F(1,28)=103.05$ $p<0.001$, drink by session by congruency, $F(1,28)=112.65$ $p<0.001$, drink by load by congruency, $F(1,28)=110.64$ $p<0.001$, session by load by congruency, $F(1,28)=78.39$ $p<0.001$ and drink by congruency by session by load $F(1,28)=82.68$ $p<0.001$. Participants were faster when presented with congruent trials at medium load and performed better two hours post interventions. Participants with elevated depressive symptoms were less accurate in both the congruent trials on both high and medium difficulty load than healthy participants, as shown in Appendix 8.

Mixed ANOVA revealed no significant effect of treatment, $F(1,28)=0.36$ $p=0.55$ or session, $F(1,28)=3.53$ $p=0.07$ on verbal fluency. Similarly, there was no significant interaction of treatment by session on verbal fluency, $F(1,28)=0.298$ $p=0.55$. Participants generated more words after the consumption of both placebo and wild blueberry drink, though this difference was not significant. Although, those with elevated symptoms of depression, produced fewer spontaneous words than those in the healthy group, this was not significant (Appendix 9).

Working memory assessed using the Keep Track Task was not significantly affected by drink, $F(1,28)=0.13$ $p=0.72$, session, $F(1,28)=0.60$ $p=0.44$ or drink by session interaction, $F(1,28)=0.18$ $p=0.61$. There was no significant change in the number of words correctly recalled before and after the consumption of placebo and blueberry drinks in both groups. Though the participants with elevated depressive symptoms recalled fewer words than the

healthy participants, the difference was not significant. Appendix 10 shows the mean words recalled before and after consumption of the interventions in both the groups.

Working memory measured using Serial 3s task was not significantly affected by drink, $F(1,28)=0.22$ $p=0.64$ nor drink by session interaction, $F(1,28)=2.87$ $p=0.10$. However, there was a significant main effect of session, $F(1,28)=6.09$ $p=0.02$, where participants performed better on the task post interventions as shown in Appendix 11. Healthy participants performed better after the consumption of blueberry drink and this change was not observed post consumption of placebo drink. Participants with elevated depressive symptoms, improved on the task after consuming both the placebo and blueberry interventions. Similarly there was no significant main effect of drink $F(1,28)=0.026$ $p=0.87$, session, $F(1,28)=2.26$ $p=0.14$ or interaction effect of drink by session, $F(1,28)=0.01$ $p=0.91$, on Serial 7s scores. As shown in Appendix 12, participants from both the groups showed improvement in their performances after the consumption of drinks. Participants with elevated symptoms of depression performed worse than those who were healthy and the change in the performance after drinks was also smaller when compared to the change in performance in healthy young adults.

Multiple Linear regression was carried out to determine the association between cognitive performance (measured in terms of accuracy, reaction time and working memory) and PHQ-9 scores. There was a significant negative correlation between the PHQ-9 scores and participants accuracy scores on incongruent trials (incongruent high load $p=0.028$ and incongruent medium load $p=0.037$), indicating that those with higher PHQ-9 scores (i.e. elevated symptoms of depression) were significantly less accurate when presented with incongruent stimuli, at both high and medium difficulty load. The regression model however was not significant, $F(10,31)=1.15$ $p=0.37$, with only 4.8% of the variance explained by the cognitive performance.

8.4 Discussion

This randomised placebo-controlled study investigated the acute effects of flavonoid rich wild blueberry drinks on transient mood, and different aspects of executive functioning in healthy university students and those who reported elevated symptoms of depression. No beneficial effects of wild blueberries were observed on transient positive or negative affect assessed using the PANAS. Though, not significant, there was an increase in positive affect after wild blueberry intervention, however, this was only observed in the healthy students. This is in line with our previous study, where a significant increase in positive affect was observed in young adults aged 18-25 and children aged 7-10, after wild blueberry consumption [(20)]. It is

important to note that in the previous study, baseline measures of depression symptoms were not taken and therefore subgroup analysis was not possible. Therefore, it is unclear if the effects on positive affect previously observed were solely in healthy young adults. Whether or not an effect of wild blueberry flavonoids on depression symptoms can be observed in those with elevated symptoms of depression warrants further investigation, as this study recruited only 12 students who reported mild to moderate depression.

Consistent with the previous study reported in this thesis within young adults and children [20], no significant effect of wild blueberry was seen on negative affect. This was also compatible with studies that investigated the effects of wild blueberry flavonoids on negative affect in children and older adults [8, 20, 68]. As expected, there was a decrease in self-reported negative affect 2 hours post drink consumption in both groups. However, the decrease seemed to be more prominent in those with elevated symptoms of depression. This may be due to the presence of sugars (i.e. carbohydrates) present in the drink. Studies have shown that dietary carbohydrates can modify plasma amino acid to enhance the uptake of tryptophan, a precursor to serotonin (one of the neurotransmitters involved in mood regulation), in the brain [69]. As the research sessions were carried out first thing in the morning, and participants were instructed to refrain from consuming food 2 hours before the test session, this may have resulted in participants skipping breakfast. If so, the drink, being a carbohydrate-rich and protein-poor meal, could have caused an increase in brain tryptophan levels, serotonin released into the synapse and a decrease in negative affect [68,69]. This change being more evident in those with elevated symptoms of depression. The sugar content in the drinks promotes insulin secretion, which is known to lower the plasma levels of other neural amino acids that compete with tryptophan for passage across the blood brain barrier. This allows for more tryptophan absorption to the brain, hence improving mood. The decrease in negative affect was more evident when wild blueberry drink was consumed. This may be due to the ability of blueberries to extend the postprandial glucose response [70], which again influences the tryptophan uptake to the brain [68]. However, this warrants further investigation in a larger group of young people with elevated symptoms of depression.

No beneficial effects of wild blueberry flavonoids were observed on executive functioning tasks. No wild blueberry related changes on reaction time or accuracy on the MANT were exhibited. As expected across both treatment groups, participants made more mistakes on the more cognitively demanding trials. Those with elevated symptoms of depression were less accurate than healthy participants. The depressed group became more accurate after consuming the drink, particularly after the placebo drink. Thus, it is plausible that the effects of the glycaemic response were more prominent in these individuals. To make the data easier

to interpret it is important to control for the glycaemic load experienced by participants when they consumed the drinks.

For incongruent trials at medium load, both groups improved in accuracy after consumption of the blueberry drink, this change however was not significant. Similarly, no intervention related changes were observed in reaction time, which was a contradiction to previous findings where wild blueberry flavonoids resulted in significantly faster reaction times without cost to accuracy in children and older adults [64,68,72]. To see if wild blueberry consumption maintains accuracy and response time, if not improve them, in cognitive tasks of varying difficulty in those with elevated symptoms of depression, warrants further investigation. No wild blueberry related effects on verbal fluency, COWATS, or working memory, KTT, serial 3s and 7s, were observed. Previously, berry juice related improvements in memory have been demonstrated in older adults [73-75], however it is important to note that in these studies there was a small sample size and age differences between the control and intervention groups, which may explain the observed effects in the Krikorian studies. Additionally, habitual intake of polyphenols was also not taken into consideration. In this study, results from previous studies regarding mood, executive functioning, verbal fluency and working memory were not replicated [11,22, 64,72,76]. This inconsistency across the studies may be due to the difference in the source of flavonoid, methodology used, and the difference in the age range of the participants, making it difficult to make direct comparisons. Additionally, previous research demonstrating various cognitive benefit of flavonoid rich foods, including short term cognitive benefit should be approached with caution as there are important implications to consider. For example, studies failing to match the placebo with the intervention drink for sugars results in the possibility that these effects are mediated by variation in blood glucose levels rather than, or in addition to, other proposed mechanism such as increased cerebral blood flow [76].

Increased glucose availability in the late postprandial period following ingestion of anthocyanin rich foods may convey a cognitive advantage and improvement in specifically defined mood states, given that low circulating glucose levels are often correlated with cognitive deficits, as foods which elicit a favourable glycaemic response, are beneficial for cognition [77], and these effects can extend beyond the first postprandial response period, continuing to influence cognition after subsequent food intake [77]. Further work is needed to investigate the effects of flavonoids on mood and cognition, making sure the effects are not masked by the glycaemic response.

In line with the study in the previous chapter, no blueberry related beneficial effects were observed on cognition, and no significant association observed between cognition and depressive symptoms. Therefore, it is plausible that improvement in cognition may not be the underlying cause of improving transient mood, at least in healthy individuals. As there was a significantly negative association between elevated depressive symptoms and accuracy scores on the more cognitively demanding tasks, improvement in cognition, due to blueberry flavonoids, may potentially be one of the underlying pathway by which transient mood is improved in those with elevated symptoms of depression. This, however, warrants further investigation with a larger group of individuals with elevated symptoms of depression and tasks that are more cognitively demanding.

8.5 Conclusion

Contrary to previous results, this study did not find significant effect of acute wild blueberry on positive affect, negative effect, accuracy, reaction time, verbal fluency and working memory, in healthy university students and those with low mood. Due to several methodological limitations including small sample size, these results should be interpreted with caution. No previous studies have investigated the effects of wild blueberry flavonoids in those with elevated symptoms of depression or those who have been clinically diagnosed with the disorder. Replication and refinement of the study methods are important, to test more accurately if there are any beneficial effects of dietary flavonoids on mood and cognition across healthy and clinical population of various age groups. If such effects on mood and cognition are observed, it would also be useful to further investigate the biological mechanisms of action.

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Chapter 9: Study 5: Effect of 4 weeks Daily Wild Blueberry Supplementation on Symptoms of Depression in Adolescents

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Revised Children's Anxiety and Depression Scale (RADS)- Appendix 13.

Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents

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Abstract

Adolescence is an important period for cognitive maturation and emotional regulation and this age group is particularly vulnerable to developing depression. Diets rich in fruits and vegetables have been associated with decreased risk of developing depressive disorders across the lifespan, an association that may be due to the high flavonoid content of these foods. Previously we have shown increases in transient positive affect in both children and young adults two hours after administration of a wild blueberry intervention. Here, using a randomised double-blind, placebo-controlled trial, we investigated the effects of four weeks, daily wild blueberry supplementation (containing ~253mg anthocyanins) on transient and chronic mood in adolescents. Healthy 12-17-year old (N = 64, 35 females) were recruited and randomly assigned to receive either a wild blueberry or matched placebo supplementation. Depression and anxiety symptoms were assessed before and after the intervention period using the Mood and Feeling Questionnaire and Revised Child Anxiety and Depression Scale. Transient affect was assessed before, two weeks, and at four weeks using Positive and Negative Affects. Following the intervention period there were significantly fewer self-reported depression symptoms in participants who were supplemented with the wild blueberry intervention compared to those who received the matched placebo ($p=0.02$, 95% CI -6.71 to -5.35). There was no between group effect on anxiety symptoms or on transient affect. Further investigation is required to identify specific mechanisms that link flavonoids consumption and mood. If replicated, the observed effects of wild blueberry supplementation may be a potential prevention strategy for adolescent depression and may have benefits for public mental health.

9.1 Introduction

Puberty is a complex biologically driven process that has an impact on emotional and behavioural wellbeing, resulting in a period with increased risk of developing emotional disorders and risk-taking behaviour. The brain undergoes cognitive maturation via synaptic remodelling well into the 20s. The limbic system, responsible for governing reward processing, appetite and pleasure seeking, matures before the prefrontal cortex, which is responsible for executive functioning such as problem solving, planning, emotional regulation, and multitasking. This difference in cortical maturity is hypothesized to create a developmental imbalance, making teens vulnerable to behavioural and mental health problems, such as depression [(1)].

An episode of major depressive disorder (MDD) during adolescence is a major personal and public health problem across the world [(2)]. The disorder has many acute and long-term adverse consequences on adolescents' education and occupational success, relationships and family life, and on their future physical and mental health [(3)]. Each year around 7.5% of adolescents aged 13 to 18 years' experience an episode of MDD [(4-6)]. Symptoms of MDD are distressing and include sleep and cognitive problems, low mood, irritability, feelings of worthlessness and lack of pleasure [(7)]. Sub-clinical MDD is even more common, recent surveys in the UK suggest that ~25% of young people report elevated symptoms of depression in any given year [(4,8)], including depressive symptoms that are not sufficient in number or severe enough to meet diagnostic criteria. Sub-clinical symptoms have a major impact on daily functioning and are associated with increased risk of developing the disorder [(4)].

Treatment for MDD in this age group includes psychological therapies and anti-depressant medication, however, these are only moderately effective and are often inaccessible to young people due to limited public health service resources [(8,9)]. A recent meta-analysis of psychological treatments for children and young people with mental health problems found that the effect size of treatment for depression was small (d = 0.29) and was lower than effects of treatment for other common mental health problems [(9)]. Many young people with MDD do not receive an evidence-based treatment and the prevention of adolescent depression is, therefore, a highly valued goal [(10)]. One potential way to prevent the onset of MDD and sub-clinical depression is through diet. Diet and depression are significantly associated in adults, although this relationship is complex and potentially bidirectional, i.e. unhealthy diet leading to low mood and vice versa [(11)]. A recent systematic review of the association between depression symptoms and diet in adolescents found that 'healthy' diets (i.e. consumption of fruits and vegetables) were associated with lower depression symptoms; whilst 'unhealthy' diets (i.e. consumption of junk foods and saturated fats) were associated with higher

depression symptoms [12]. A large well-controlled epidemiological study examining associations between habitual intakes of dietary flavonoids and depression risk showed that individuals consuming diets higher in flavonoids presented a lower depression risk, particularly amongst older women [13]. A similar study assessed symptoms of depression and the total habitual intake of polyphenols among the participants and found that higher dietary intake of flavonoids was inversely associated with depressive symptoms [14]. Thus, diets rich in fruits and vegetables are associated with low depression symptoms. Dietary flavonoids are present in substantial concentrations in commonly consumed fruits and vegetables and may be a potential mediator for the anti-depressant action of diets rich in fruits and vegetables.

The hypothesis that there is a causal relationship between diet and depression symptoms and the onset of MDD has recently been strengthened by number of intervention studies. Acute purple grape juice intervention resulted in increase in self-reported ratings of 'calm' in healthy young adults [15]. Similarly, acute consumption of flavonoid-rich wild blueberry improved short-term positive mood in children aged 7-10 years and in young adults aged 18-25 years [16]. In a recent randomised controlled trial with 67 depressed adults [17], participants randomised to an intervention promoting a healthy diet with at least nine portions of fruits and vegetables each day reported significantly less depression at twelve weeks than those randomised to receive social support. Anti-depressive effects of flavonoid rich plants and their extracts have also been investigated. *Hypercium perforatum* (also known as Saint John's wort, derived from a flowering plant in the Hypericaceae family) extract intervention studies show its effectiveness as treatment for mild/moderate depression when compared to placebo and having similar effects to pharmacological treatments [18-21]. Similarly, saffron (*Crocus sativus*, derived from the saffron spice of the flowering plant of Crocus genus) extract consumption had equivalent effect as pharmacological treatment for depression and was significantly more effective than the matched placebo [22-24].

The specific effects of sustained wild blueberry flavonoid consumption on symptoms of depression in adolescents have not yet been tested. Here, we designed a double-blind, placebo-controlled experiment to test the effect of consuming a flavonoid-rich wild blueberry intervention for four weeks on symptoms of depression, anxiety, and transient affect in healthy adolescents. Participants were randomly assigned to a wild blueberry or a matched placebo drink with transient affect and symptoms of depression and anxiety assessed before and after the four-week intervention period.

9.2 Methods

***9.2.1 Ethics*:** This research was reviewed and given a favourable ethical opinion for conduct by the University of Reading Research Ethics Committee (UREC 16/55). The study was registered at clinicaltrials.gov NCT03119597.

9.2.3 Participants: An *a priori* power analysis (using G Power 3.1.9.2) based on data from a previous study [16] revealed that 24 participants per group were required to achieve power of 0.8 with alpha set at 0.5 level. Students aged 11-17 years of varying ethnicity, from four schools in Reading Berkshire, UK were invited to take part in this study. All parents or legal guardians provided informed written consent for young people under the age of 16. Participants under the age of 16 provided written assent and those over 16 gave written consent. All participants were screened for any health conditions (including mental health), any treatment they were receiving and food related allergies that would exclude them from the study. We screened 82 young people, of whom 18 dropped out after the first screening session. Sixty-four participants were randomly assigned to either a wild blueberry drink or a matched placebo drink. The randomised allocation of participants to treatment was generated using excel. The groups were coded A and B and the sequence was saved in a password protected spreadsheet. Both the researchers and the participant were blind to treatment group and participants were told the study was investigating effects of different fruit drinks so were not aware of the study hypothesis.

9.2.4 Interventions: Both interventions (wild blueberry and placebo) were measured and packaged into silver opaque sachets at the University of Reading. Sachets were identical for the wild blueberry and the placebo drink and neither the researchers nor the participants knew what their sachets contained. Wild Blueberry Association of North America (WBANA) provided the blueberry powder and the matched sugars and vitamin C (placebo) was obtained from Bulk Powders. The packets of wild blueberry contained 13g of freeze-dried wild blueberry (WBB) powder (containing ~253mg anthocyanins). Placebo packets were matched to the WBB for sugars (4.52g glucose and 4.79g fructose) and vitamin C (4 mg). Analysis of anthocyanin content was carried out by independent researchers from WBANA indicating anthocyanin content of 12.44 mg/g which, given a freeze dried to fresh ratio of 7/1, is equivalent to 177.7mg/100g fresh (see Table 1). Each participant was given 14 days' supply of their requisite intervention, along with written and video instructions for their parents/guardians on how to prepare the intervention. Each intervention was prepared daily, by adding 30 ml of low flavonoid 'Rock's Organic Orange Squash' (containing 13.2mg total polyphenols Narirutin & Hesperidin) and 170 ml of water and the contents of the sachet to the opaque cup provided. Each participant was given a checklist to record the dates and times

when they consumed the drink each day and the name of the person who prepared the drinks. Participants were also asked to bring back their used sachets after two weeks as a measure of compliance. The remaining 14 days' supply of each intervention was given to the participants two weeks into the intervention period. The true aim of the study was not disclosed to the participants, they were informed that it was a fruit drink study, to avoid revealing the identity intervention of the drink.

Table 1. Showing the anthocyanin content of the intervention drink.

	mg/g freeze dried	mg/ 100 g fresh BB
Delphinidin	3.67	52.42
Cyanidin	1.69	24.17
Petunidin	2.28	32.57
Peonidin	0.74	10.00
Malvidin	4.07	58.14
Total	**12.44**	**177.7**

9.2.5 Measures:

The Mood and Feelings Questionnaire (MFQ) was used to measure symptoms of depression [(25)]. The MFQ is considered to be the gold standard self-report measure for depression in young people (NICE, 2015). It is a standardized and well-validated 33-item self-report measure of the severity of depression symptoms in adolescents. Each item relates to a symptom or experience associated with depression. Participants are asked to rate each item in relation to their symptoms in the past 2 weeks on a 3-point Likert scale (not true = 0, sometimes = 1, true = 2). Total MFQ scores range from 0 to 66 where higher scores indicate greater risk of depression. The clinical cut off for the MFQ is 27, with scores above 27 indicating significant risk of a diagnosis of MDD [(25)].

Anxiety symptoms were assessed using the anxiety sub-scale of the Revised Child Anxiety and Depression Scale (RCADS) [(26)], a standardized and validated measure of anxiety symptoms in young people, used routinely in UK NHS mental health services. The anxiety sub-scale of RCADS consists of 37 items, each rated on a 4-point Likert scale (never =1, sometimes = 2, often = 3, always = 4). Total scores range from 37 to 148 with higher scores indicating increased risk of an anxiety disorder. Again, participants were asked to rate the items keeping the past two weeks in mind.

Current mood (i.e. transient affect) was assessed using the Positive and Negative Affect Schedule-NOW (PANAS-NOW) at screening, and at two and four weeks. As the term suggests this is a measure of how the participants felt at that moment in time (transient mood).

The PANAS is a valid and reliable 20 self-report measure of positive affect (PA – 10 items) and negative affect (NA - 10 items) that can be used on multiple test occasions [27,28]. Participants rated the degree to which they were currently experiencing each item on a 5-point Likert scale ranging from 'very slightly' to 'extremely'. Ratings of positive and negative items were summed to calculate an overall positive affect and overall negative affect score, each ranging from 10-50 where lower scores indicate lower levels of positive or negative affect.

Habitual fruit and vegetable consumption were assessed using EPIC-Norfolk food frequency questionnaire, a semi-quantitative paper-based questionnaire, which includes 130 food items, each rated on 9-point Likert scale (never or less than a month-1 to 6+perday-9). FETA software was used to analyse the data collected to calculate 46 nutrient and 14 food group values including average daily fruit and vegetable intake [29].

9.2.6 Procedure: As outlined in Figure 1, participants were seen by the researchers four times across a five weeks period. All participants did not attend all assessment – the number of participants assessed at each timepoint is indicated in Figure 1. Research sessions took place either at the University of Reading or at the participant's school. Sessions were scheduled at the same time of day for each participant. The first two sessions, scheduled 48 hours apart, were screening sessions where participants completed a battery of questionnaires: MFQ, RCADS (screening session 1), PANAS, EPIC- Norfolk food frequency questionnaire and a questionnaire about their health status (screening 2). Screening sessions were limited to 30 minutes to fit with the school timetable and to maintain high levels of participant engagement in both sessions. Parents were also asked to complete a demographic questionnaire. Participants started the intervention the day after the second screening session was completed. Two weeks later they returned their used drink sachets, were given a new checklist and completed the PANAS to maintain their engagement (Test session 1). Participants were instructed not to consume the intervention prior to the test session to ensure chronic effects of the intervention on transient were measured. Participants were also asked if they were experiencing any adverse effects of the drink and feedback on its palatability. They then returned two weeks later (Test session 2), returned their drink sachets, completed the PANAS, MFQ and RCADS and were debriefed. For each test session, participants were instructed not to consume their allocated intervention before the test session to ensure that chronic, not acute, effects of the intervention were being measured.

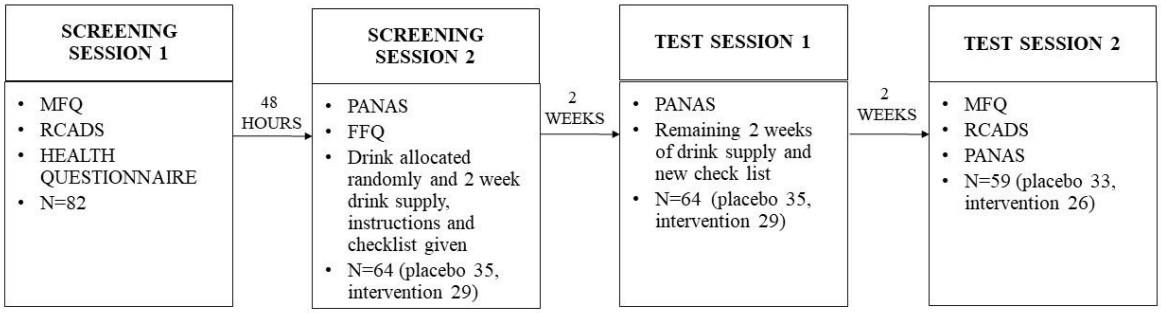

Figure 1. A schematic of the study of the study procedure

9.2.7 Statistical Analysis: Statistical analyses were conducted using IMB SPSS version 22. Any differences in symptoms of depression, anxiety and intake of fruits and vegetables between the two groups at baseline was investigated using T-test. Effects of intervention on transient affect was analysed using Linear Mixed Modelling (LMM) using an unstructured covariance matrix to model successive repeat test sessions, with subjects included as random effects. Data from two weeks and four weeks measures of the PANAS and treatment group were included as fixed factors, with baseline PANAS scores included as a covariate. LMM deals with data that is missing at random and with multiple measurement points, giving unbiased estimates of each of the means. To test the effects of the intervention on anxiety and depressive symptoms at four weeks, data were analysed using Analysis of Covariance (ANCOVA) with drink (Placebo, WBB) as an independent variable and MFQ and RCADS scores at 4 weeks as dependent variable. Baseline measures of depression and anxiety were used as covariates and Bonferroni corrected T-tests were used to investigate all fixed effects and interactions.

9.3 Results

9.3.1 Sample characteristics:

Sixty-four participants were randomised (35 females, 29 males), aged 12-17 years (M = 14.20 years, SD = 1.71). Thirty-five participants were randomly allocated to receive the placebo drink and twenty-nine to the WBB intervention. Participants' demographic data, baseline mood scores and habitual fruit and vegetable intakes are reported in Table 2. There were no significant differences between groups in the amount of daily fruit t (51) = 0.14, p = 0.89 or vegetables t (51) = 1.45, p = 0.15 consumed. One sample t-test revealed that the mean fruit

and vegetable consumption by the participants was significantly lower than the 400g per day as recommended by WHO; fruit: t (52) = 11.20, $p<0.005$, vegetables t (52) = 7.12 $p< 0.005$).

Table 2: Demographic details, mean fruit and vegetable intake and mean depression and anxiety scores at baseline for both intervention groups.

	PLACEBO GROUP	WILD BLUEBERRY GROUP	P VALUES
MEAN AGE	14.5 (SD=1.804)	13.82(SD=1.54)	P=0.11
MALE %	48.6	41.4	P=0.57
FEMALE %	51.4	58.6	P=0.57
BRITISH %	60	52.4	P=0.52
ASIAN%	11.4	12.5	P=0.52
MIXED%	5.8	12.6	P=0.52
AFRICAN	2.9	8.3	P=0.52
CHINESE	2.9	4.2	P=0.52
MEAN FRUIT INTAKE (GRAMS/DAY)	188 (SD=168.3)	176 (SD=98.0)	P=0.89
MEAN VEGETABLES (GRAMS/DAY)	257.6 (SD= 187.0)	187.5 (SD=144.6)	P=0.15
MEAN DEPRESSION (MFQ)	13.0 (SD= 10.0)	11.3 (SD= 8.5)	P=0.55
MEAN ANXIETY (RCADS)	24.2 (SD= 14.90)	22.3 (SD=13.0)	P=0.66
MEAN POSITVE AFFECT	28.0 (SD=7.7)	25.3 (SD=8.0)	P=0.17
MEAN NEGATIVE AFFECT	15.1 (SD=5.24)	14.1 (SD=4.38)	P=0.98

At baseline mean depression and anxiety scores were 12.35 (SD = 9.31) and 23.19 (SD = 13.80) respectively, both below the clinical threshold. There was no significant group difference in symptoms at baseline; MFQ, t (60) = 0.60, p = 0.55, RCADS, t (40) = 0.45, p=0.66 and no group difference in mean positive and negative affect; t (62) = 1.40, p=0.17 and t (62) =0.80, p=0.98 respectively. A minority of participants (9.38%) reported depression symptoms above the clinical cut-off of 27 on the MFQ (11.4% in the placebo group, 3.4% in the intervention group) as shown in Table 3. No participants reported anxiety symptoms above the clinical threshold. No participants reported a diagnosis of depression or anxiety, or that they were receiving treatment for these disorders.

Table 3. showing the number of male and females with and without symptoms of depression.

	Healthy (MFQ score < 14)	**Elevated Symptoms (MFQ score score >27)**	**Total**
Male	22	7	29
Female	27	8	35
Total	49	15	64

9.3.2 Hypothesis testing*:* At four weeks 59 participants provided self-report data on anxiety (RCADS) and depression (MFQ) symptoms; 26 from the intervention group and 33 from the placebo group. As shown in Figure 2a, after four weeks of the intervention, the mean MFQ score for participants who consumed WBB was significantly lower than the mean MFQ score for participants who consumed the placebo drink. This was significant F(1,57)=5.52, p=0.02 95%CI -6.71 to -5.35 with a medium effect size (d = 0. 65). The change in the depression scores for each participant including regression line for both treatments is shown in Figure 3. There was no significant effect of WBB on symptoms of anxiety (Figure 2b) after four weeks of supplementation F (1,34) = 2.1, p=0.16; mean RCADS score for participants in the WBB group was 13.90, (SD = 8.39) and the mean RCADS for the placebo group was 19.3, (SD = 11.31).

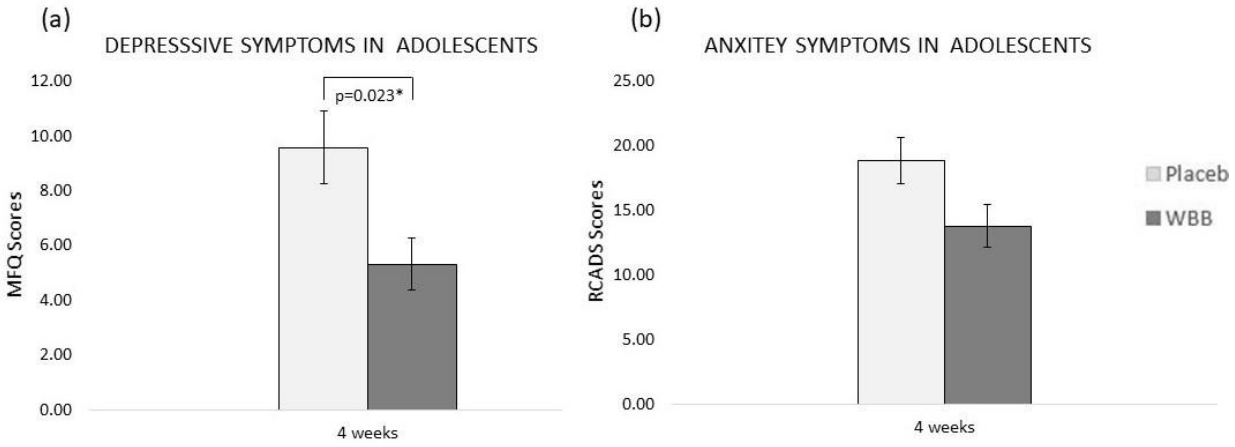

Figure 2. Mean scores (± standard error of the mean) in adolescents aged 11-17 years (a) Mean MFQ scores after 4 weeks consumption of placebo and intervention drinks. (b) Mean RCADS scores after 4 weeks consumption of placebo and intervention drinks.

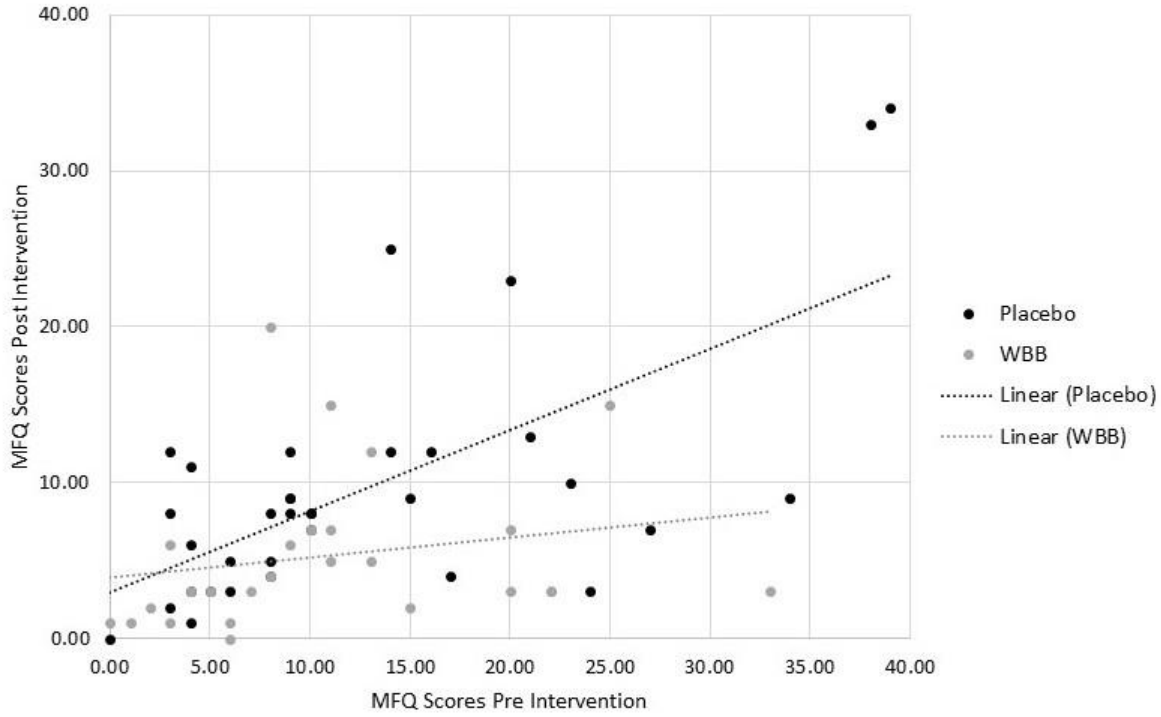

Figure 3. Scatterplot showing the MFQ scores at baseline and 4-week post intervention

We also examined the effect of intervention on positive affect and negative affect (PANAS) after two and four weeks (see Figure 4). There was no significant effect of Drink ($F(1,64.33)=0.26$ $p=0.62$), Repeated trial , ($F(1,62.22)=2.95$ $p=0.09$), or any Drink x Repeated trial interaction ($F(1,62.22)=3.686$, $p=0.06$) on transient positive affect. As shown in Figure 4a, mean PA scores following intervention of WBB drink at two weeks was 25.55(SD= 9.71) and at four weeks was 23.04(SD= 8.07) and following the placebo drink at two weeks was 25.86 (SD=7.69) and at four weeks 26.30 (SD=7.54). There was also no significant effect of the intervention on NA; Repeated trial, $F(1,59.3)=0.66$ $p=0.42$, Drink, $F(1,63.79)=0.24$ $p=0.63$ or Repeated trial × Drink interaction, $F(1,59.30)=1.17$ $p=0.28$. As shown in Figure 4b, NA was not significantly different after consuming the WBB drink (2 weeks: M(SD) = 13.69(4.02) and 4 weeks: M(SD)=13.19(5.55) or the placebo (2 weeks: M(SD) = 13.4(3.96) and 4 weeks: M(SD)=14.24(5.24)).

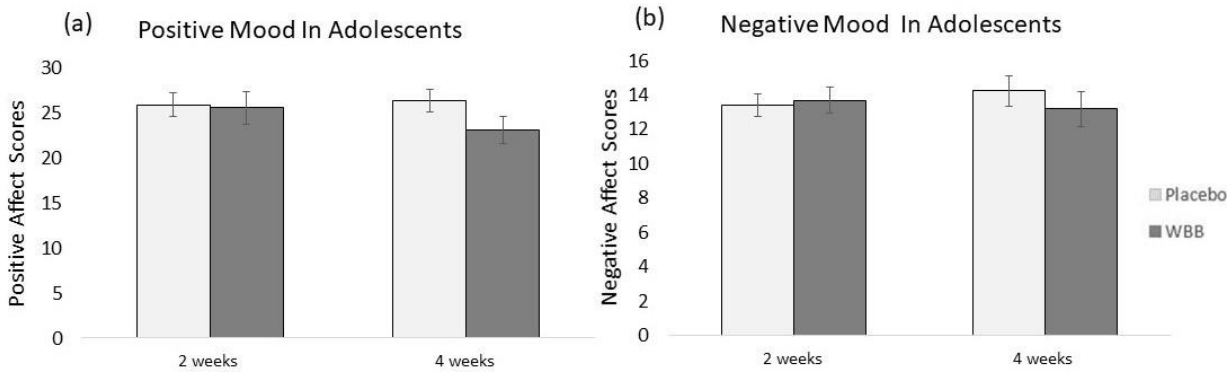

Figure 4. Mean PANAS-NOW Mood scores (± standard error of the mean) in adolescents aged 11-17 years: (a) Mean PA scores 2- and 4-weeks post-consumption of placebo and intervention drinks. (b) Mean NA scores 2- and 4-weeks post-consumption of placebo and intervention drinks.

9.4 Discussion

This randomised, placebo controlled, double blinded trial investigated the effects of 4 weeks of consumption of a flavonoid-rich WBB drink on symptoms of depression and anxiety and on transient affect in a community sample of healthy 12-17-year old. The results demonstrated that after four weeks of daily WBB intervention there was a between groups difference in self-reported depressive symptoms; participants randomised to the WBB intervention reported significantly lower scores on the measure of depression symptoms than participants who were randomised to the placebo drink. There was no significant effect of the intervention on anxiety symptoms or on PA and NA (transient affect). The data suggest that flavonoid supplementation may be beneficial in reducing depressive symptoms in healthy adolescents.

This is, to our knowledge, the first randomised double blinded study to show the effects of chronic WBB flavonoids on depression symptoms in teenagers. The participants in the study were healthy but at baseline assessment were consuming sub-optimal habitual levels of flavonoids, i.e. daily consumption of fruit (44.87%) and vegetable (57.46%) was well below the WHO recommended amount of 400g/day [30,31]. This is consistent with the typical diet of young people in the UK, where only 18% of adolescents meet the recommended daily requirement, and the average daily consumption within this age group is 256g (3.5 portions) of fruit and vegetables [32]. Levels of depression and anxiety were similar to community norms gold standard self-report measures. Importantly, because the effects of the intervention were observed in a community sample, these effects cannot necessarily be generalised to adolescents with more severe symptoms of depression or a diagnosis of depression.

Within this community sample, the effect size of the flavonoid intervention compared to the control group on the measure of depression symptoms, the MFQ, was d = 0.65, a medium effect size. To put this into context, two recent meta-analyses have examined the effects of psychological treatments for depression and the prevention of depression. Ecksthtain et al., (2019) concluded that the treatment effect size of psychological treatments for adolescents with depression was d = .36 [33]. In a review of interventions to prevent depression Ssegonia et al., (2019) reported an effect size of d = .22 [34]. In relation to the specific measure of depression used in this study, the reduction of the 4 points on mean MFQ scores in the intervention group indicates a complete amelioration of 2 items on the scale or a reduction (from 2 to 1, or 1 to 0) of 4 items. Because each item reflects a symptom or adverse effect of depression, clinically this would be likely to reflect a meaningful reduction in the impact of depression on the young person [35].

Previously the effects of flavonoids from different sources such as apples, cocoa and grape juice showed no effects on depression in healthy adults [36-39]. However, our results are consistent with previous animal and epidemiological studies that suggest anti-depressive effects of a flavonoid rich diet [13,40-43].They also are in keeping with experimental data on the acute effects of WBB on positive mood in children and young adults [15,16], and the acute effect of grape juice on mood in healthy adults. [44]. Unlike a previous acute intervention study, we did not observe a significant effect of WBB on momentary mood (i.e. transitory affect). However, the interval between consuming the WBB drink and assessing NA and PA was variable, unlike the standard 2-hour interval used in previous studies. In addition, the four-week assessment (our end point) was conducted during the first week of school after the summer holidays. Unlike symptoms of depression (and anxiety) which were measured over a minimum two-week period and which are conceptualised as relatively stable, positive and negative affect are conceived as short-lived events that have rapid decay after elicitation [45]. It is therefore possible that this external event (returning to school) had a measurable impact on participants' momentary affect.

Although anxiety and depression are frequently co-morbid in young people and share some symptoms (e.g. fatigue, low concentration and sleep disturbances), the results of this intervention study suggest that flavonoids may reduce symptoms that are more prominent in depression than anxiety, e.g. low mood, anhedonia, feelings of guilt, and worthlessness and do not reduce symptoms that are specific to anxiety. It is also possible that the effect of flavonoids on anxiety is smaller than the effect on depression and that a larger sample, with greater power, might result in a significant effect.

Some authors have proposed that flavonoids increase cerebral blood flow to the dorsolateral prefrontal cortex, a site that is highly associated with cognitive and emotional regulation, including rumination, a cognitive process of repetitive thinking that may exacerbate feelings of guilt and worthlessness [46-48]. This suggests that there may be an indirect pathway between flavonoid consumption and depression whereby flavonoid consumption enhance cerebral blood flow, which boosts executive functioning; in turn improved executive functioning helps to enhance cognitive control, inhibits rumination, and thus reduces depression. Adolescents with depression have impaired executive function compared to non-depressed and anxious young people [49] and therefore the benefits of flavonoid consumption may be more prominent in these young people. However, potentially any positive effects of flavonoid consumption on executive function would have benefits for more young people because executive function is critical for academic achievement [50].

A plausible direct pathway between flavonoid consumption and mood is the effects of flavonoids on Monoamine Oxidase (MAO). MAO inhibitors have been used to treat mood disorders and flavonoids may mimic their effects [51,52]. A recent study showed that consuming fruits high in flavonoids i.e. blackcurrants significantly reduces MAO activity and increases the circulating monoamines and thereby elevates mood [51]. Another possible mechanism by which flavonoids may affect mood is by mimicking anxiolytic-like effects by binding to benzodiazepine receptors, enhancing the effect of GABA via GABAA receptors [33,53,54]. However, in line with a previous study [16] that showed no changes in negative affect (an indicator of anxiety) after acute flavonoid intervention, here there was no significant of flavonoid consumption on anxiety.

Although the mechanisms of action require further investigation there is accumulating evidence of a causal relationship between flavonoid consumption and depression symptoms. This evidence has been published by independent research groups using different research designs, including epidemiology, clinical trials, and experiments. However, the research is preliminary and requires robust replication and extension, with larger samples, longer time scales and careful tests of mechanisms of action. Our study examined the effects of flavonoids on healthy young people, some of whom had elevated symptoms of depression. We did not have adequate power to conduct sub-group analysis but clearly it is important to identify if the change in depression symptoms is driven by improvements in those with relatively elevated symptoms, or if the effects are similar across all levels of baseline depression. This distinction is important because flavonoids may have the potential to prevent depression in those at risk (i.e. those with elevated symptoms) or may have a more general effect. The former would suggest that dietary interventions could be used for early intervention in those

exhibiting symptoms of depression; the latter that dietary interventions could have a broader benefit to public mental health.

9.5 Conclusion

This randomised double-blind study demonstrated the chronic effects of wild blueberry flavonoid consumption on reducing symptoms of depression in a community sample of adolescents. Dietary flavonoid interventions may have potential to reduce symptoms of depression in adolescents. This study requires replication, not only in healthy participants, but also in clinically referred samples to assess the potential of flavonoids to be used as a practical and cost-effective intervention. In addition to this, studies focused on investigating biochemical changes and investigating the mechanistic pathways in which flavonoids decrease depressive symptoms in humans is essential.

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Chapter 10. Addendum to Study 5: Effect of 4 Weeks Daily Wild Blueberry Supplementation on Cognition in Adolescents

10.1 Introduction

In this double-blinded, placebo-controlled study, we also explored the effects of four-week blueberry intervention on cognition in addition to sustained and transient mood. Previously (Chapter 7) we examined the effects of flavonoid supplementation on cognition in young adults, including, response interference assessed using the Modified Attention Network Task. There was no significant difference in participants' accuracy and response times for either congruent or incongruent trials two hours after acute supplementation of wild blueberry flavonoids.

The effects of blueberry flavonoids have also been explored on other cognitive domains that are impaired during periods of depression, including verbal fluency and working memory. In young adults we did not find any effect of blueberry flavonoids on these cognitive processes (Chapter 8). However, the research evidence is inconsistent. Not only different areas of cognition are tested, such as visuospatial working memory, executive function, but also the interval between consumption and testing differs from study to study. For instance, Whyte, Schafer & Williams (2016) showed significant improvements in immediate word recall after 1.25 hours, delayed word recognition at 6 hours and accuracy on a Flanker task after 3 hours of wild blueberry consumption [(1)]. Bondonno et al., (2014) found no significant effect of flavonoid from apples on working memory or attention 2.5 hours post supplementation [(2)]. Further investigations therefore need to be carried out to determine the effects of flavonoids on cognition between different age groups, using different flavonoid sources, doses, and intervals between consumption and testing to build up a robust evidence base on the effects of flavonoids on cognitive processes [(3)].

Another cognitive process which is disrupted in major depressive disorder is rumination. Rumination is a process of uncontrolled, narrowly focused, negative thinking that is often self-referential and lies at the core of depression [(4)]. Rumination is usually accompanied by other cognitive deficits including over general autobiography memory in which details of autobiographical history cannot be recalled. It is hypothesised that ineffective cognitive control over emotional information, accompanied by increased emotional reactivity to negative self-referential stimuli, underlie depressive rumination [(5)]. The main neuronal basis for tendency to ruminate is the hyperactivation of the dorsolateral prefrontal cortex (DLPFC) and its ineffective modulatory actions on the temporal areas of the brain in response to emotional stimuli [(5)]. A study investigating the neural effects of green tea flavonoid showed

and increased activation of DLPFC in adults post flavonoid intervention [6]. However, the effects of flavonoids on rumination has not been investigated.

There are a limited number of studies that have examined the effects of flavonoids on mood and cognition in children and adults, but we are not aware of any research with adolescents or looking specifically at rumination. Puberty is a complex biologically driven process that has an impact on emotional and behavioural wellbeing, resulting in a developmental period (adolescence) when there is an increased risk of developing emotional disorders and of risk-taking behaviour. The brain undergoes cognitive maturation via synaptic remodelling well into the 20s. The limbic system, which is responsible for governing appetite and pleasure seeking, matures before the prefrontal cortex which is responsible for executive functioning such as problem solving, planning, emotional regulation, and multitasking. This creates a developmental imbalance, making teenagers vulnerable to develop behavioural and mental health problems, such as depression and anxiety [7].

This addendum to the previous chapter, aims to investigate the effects of wild blueberry flavonoids on further cognitive domains such as rumination and working memory, in addition to assessing attention and response interference in adolescents.

10.2. Methods

In addition to the mood measures used in the previous study (Chapter 9) cognitive performance was assessed using the following measures:

Modified Attention Network Task (MANT) [1]: Selective attention and response times was measured using MANT. Participants' accuracy and response time were measured as baseline two and four weeks after blueberry or matched placebo intervention. White arrow symbols "<" and ">", 5 in a row, were presented on a black background. Stimulus (i.e. middle arrow) was either congruent (>>>>> or <<<<<) or incongruent (<<><< or >><>>) with pairs of arrows on either side. Stimuli were presented in two difficulty settings, medium load, where only one row of arrows were presented and high load, where two rows of arrows were displayed. Presentation was randomised so the probability of congruent and incongruent stimulus was equal. Pseudorandom stimulus interval of 1000, 1300 or 1500ms was presented following the stimulus which was displayed for 120ms. Participants were required to press the left and right arrow keys on the keyboard to indicate the direction of the stimuli arrow. Any response times less that 100ms were removed as it may have been intended for the previous stimuli.

Keep Track Task (KTT) [8,9]: Working memory was measured using KTT. It assesses how participants update and modify the information retained. They were presented with set of categories (animals, colour, furniture, relatives, and fruits) and words from these categories are presented sequentially, 2-3 words from each category. Participants were required to remember and write down the last (most recent) word presented from each of the target category at the end of each trial. The proportion of correctly identified words was calculated. This was again assessed at baseline and two- and four-weeks post intervention.

Rumination Response Scale (RRS) [10] (Appendix 14): The RRS was used to measure ruminative tendencies. This is a 22-item questionnaire commonly used with adolescents that has a strong psychometric property [11]. The items assess two aspect of rumination, brooding and reflective pondering by measuring the coping strategies that an individual may use whilst facing low mood such as finding a meaning by ruminating, repetitively paying attention to the symptom and trying to Figure out the possible causes or consequences of the actual emotional state. Participants were asked to rate each item on a 4-point Likert scale (1=almost never, 2=sometimes, 3=often, 4=almost always). The ratings were then summed up to calculate the total rumination score ranging from 22 to 88, with higher scores indicating higher rumination. Participants' rumination was assessed at baseline and four weeks after the intervention.

Analysis: The effects of the chronic flavonoid intervention on cognition was analysed using Linear Mixed Modelling (LMM) using an unstructured covariance matrix to model successive repeat test sessions, with subjects included as random effects. Data from two-weeks and four-week measures of cognition (i.e. Flanker task, COWAT and KTT) and treatment group were included as fixed factors with baseline scores of all the cognitive measures included as covariates.

To test the effects of the chronic intervention on rumination, data was analysed using Analysis of Covariance (ANCOVA), as only a single time point (post 4-week intervention) was analysed, with Drink (Placebo, wild blueberry) as independent variable and Rumination scores as dependent variable. Baseline measures of rumination were used as covariates. Bonferroni corrected t-tests were used to investigate all fixed effects and interactions.

The correlation between depressive symptoms and cognition at baseline was assessed using multiple linear regression with MFQ scores as the criterion variable and cognitive measures (accuracy, reaction time, working memory, verbal fluency, and rumination) and age as predictor variables.

10.3 Results

There were no significant differences in rumination scores at baseline between the two treatment groups (p>0.05).There was no significant effect of wild blueberry on rumination after 4 weeks of supplementation, F(1,55)=0.17, p=0.69. Mean rumination score for participants in the wild blueberry group was 31.56, (SD = 9.13) and the mean rumination for the placebo group was 34.58, (SD = 10.76) as shown in Figure 1.

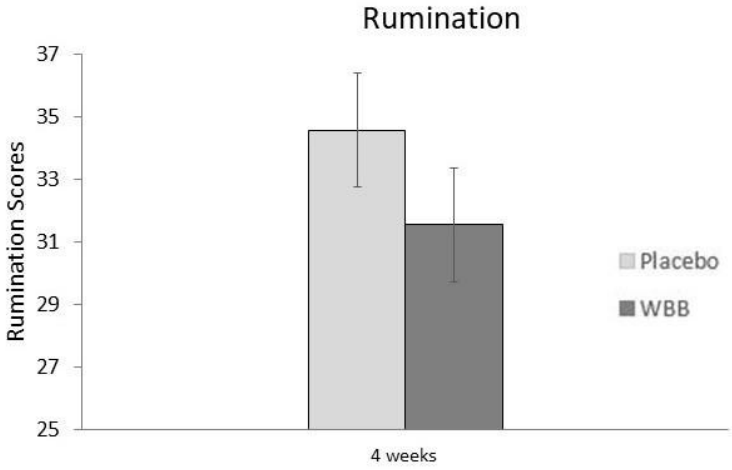

Figure 1. Mean (± standard error of mean) rumination scores in adolescents aged 11-17 years after 4 weeks consumption of placebo and intervention drinks

The effects of chronic wild blueberry supplementation on working memory, assessed using the KTT, was analysed using LMM. There was no significant main effect of baseline, revealing no differences in KTT scores between groups at baseline. There was no significant effect of Drink (F(1,59.51)=0.83 p=0.37), Session (F(1,59.5)=2.21 p=0.14) or Drink x Session (F(1,59.52)=1.6 p=0.211).

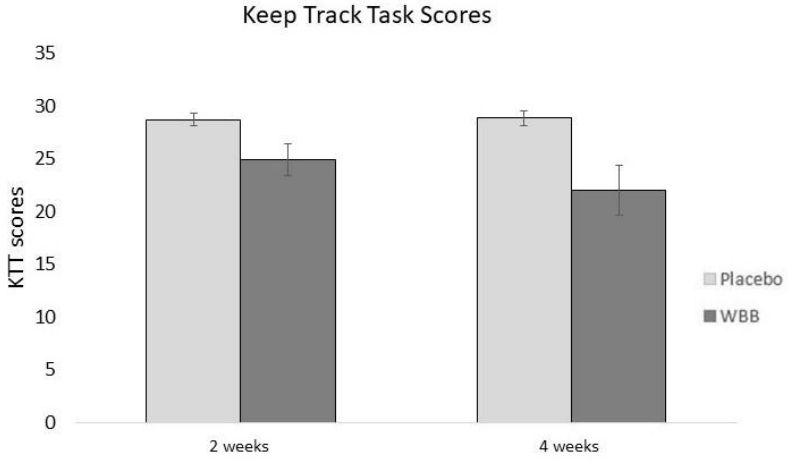

Figure 2. Mean (± standard error of mean) Keep Track Task (KTT) scores at 2 weeks and 4 weeks post-consumption of placebo and intervention drinks in adolescents aged 11-17 years.

LMM was also used to investigate the effects of the intervention on executive functioning by assessing participants response time and accuracy on the MANT. No significant main effect of baseline accuracy or reaction time was observed for congruent and incongruent trials at different levels of cognitive demand ($p>0.05$). The mean accuracy scores for congruent and incongruent trials at different levels of cognitive demand are presented in Figure 3. There was no significant main effect of session ($F(1,511)=2.49$ $p=0.115$) on participants accuracy on the MANT. However, there was a significant main effect of drink ($F(1,511)=1.17$ $p=0.001$), congruency ($F(1,511)=12.47$ $p<0.05$) and load ($F(1,511)=31.32$ $p<0.005$), where participants who were assigned to the placebo group (M(SD)=0.88 (0.29)) were more accurate than those in the blueberry group (M(SD)=0.83 (0.22)). Also, participants in both groups were more accurate when presented with a medium load (M(SD)=0.89(0.26)) than with a high load (M(SD)=0.82(0.27)) and when congruent stimuli (M(SD)=0.88(0.23)) were presented compared to incongruent stimuli (M(SD)=0.83(0.28)).

There was no significant interaction of drink x session ($F(1,511)=0.14$ $p=0.71$), session x congruency ($F(1,511)=0.15$ $p=0.70$) or session x load ($F(1,511)=0.08$ $p=0.77$) on accuracy on the MANT. However, there was a significant interaction of drink x congruency ($F(1,511)=14.93$ $p<0.005$), drink x load ($F(1,511)=16.49$ $p<0.005$), session x congruency x load ($F(2,511)= 5.47$ $p=0.004$) and drink x session x congruency x load ($F(4,511)=5.04$ $p=0.001$). Pairwise comparisons revealed that those assigned to placebo (M(SD)=0.88(0.299)) were more accurate when presented with incongruent stimuli than those assigned to wild blueberry drink (M(SD)=0.79(0.245)). Likewise, there was significant difference in the accuracy score between those who were assigned to the placebo (M(SD)=0.86(0.29)) and those who received the blueberry drink (M(SD)=0.77(0.24)), when presented with high load stimuli. There was also a significant difference in the accuracy scores in participants who received the wild blueberry drink when presented with high (M(SD)=0.77(0.24)) and medium (M(SD)= 0.89(0.21)) load.

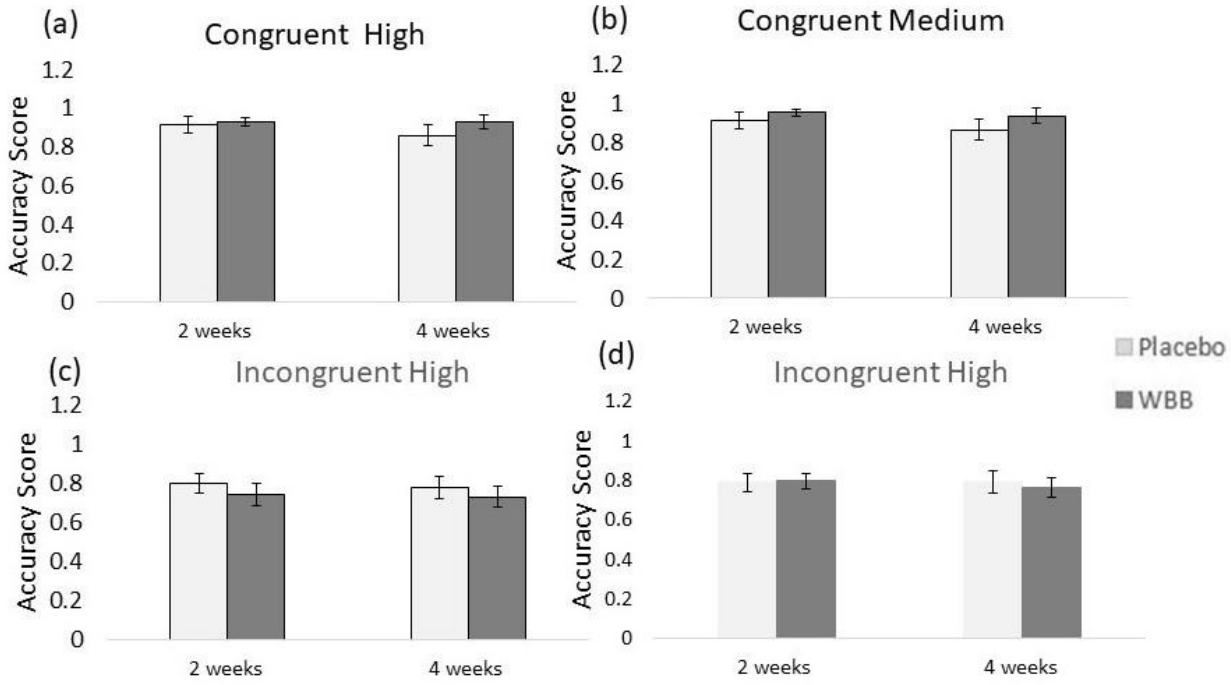

Figure 3. Mean (± standard error of mean) Accuracy score on MANT 2- and 4-weeks post-consumption of placebo and intervention drinks in adolescents aged 11-17 years: (a) Mean Accuracy score when congruent/high stimuli were presented. (b) Mean Accuracy score when congruent/medium stimuli were presented. (c) Mean Accuracy score when incongruent/high stimuli were presented. (d) Mean Accuracy score when incongruent/medium stimuli were presented.

There was no significant main effects of drink, $F(1,512)=0.42$ $p=0.52$ or Load, $F(1,512)=0.02$ $p=0.88$, on participants' response time. However, there was a significant main effect of session and congruency, $F(1,512)=26.01$ $p<0.005$ and $F(1,512)=0.022$ $p=0.018$ on response time. Participants' reaction time on the Flanker Task significantly decreased over, time 2-week M(SD)=463.04(75.92) and 4-week M(SD)=415.49 (144.04)). Participants were also significantly faster when presented with congruent stimuli (M(SD)=427.35(103.75) when compared to incongruent stimuli (M(SD)=451.17 (123.89). There was no significant drink x session ($F(1,512)=0.11$ $p=0.75$), drink x congruency ($F(1,512)=0.11$ $p=0.811$), drink x load ($F(1,512)=0.057$ $p=0.81$) session x congruency ($F(1,512)=0.43$ $p=0.52$), session x load ($F(1,512)=0.005$ $p=0.95$), session x congruency x load ($F(2,512)=0.22$ $p=0.80$ and dink x session x congruency x load ($4,512)=0.22$ $p=0.93$) interaction effect of intervention found on reaction time. The mean reaction times when presented with congruent and incongruent stimuli at high and medium load is presented in Figure 4.

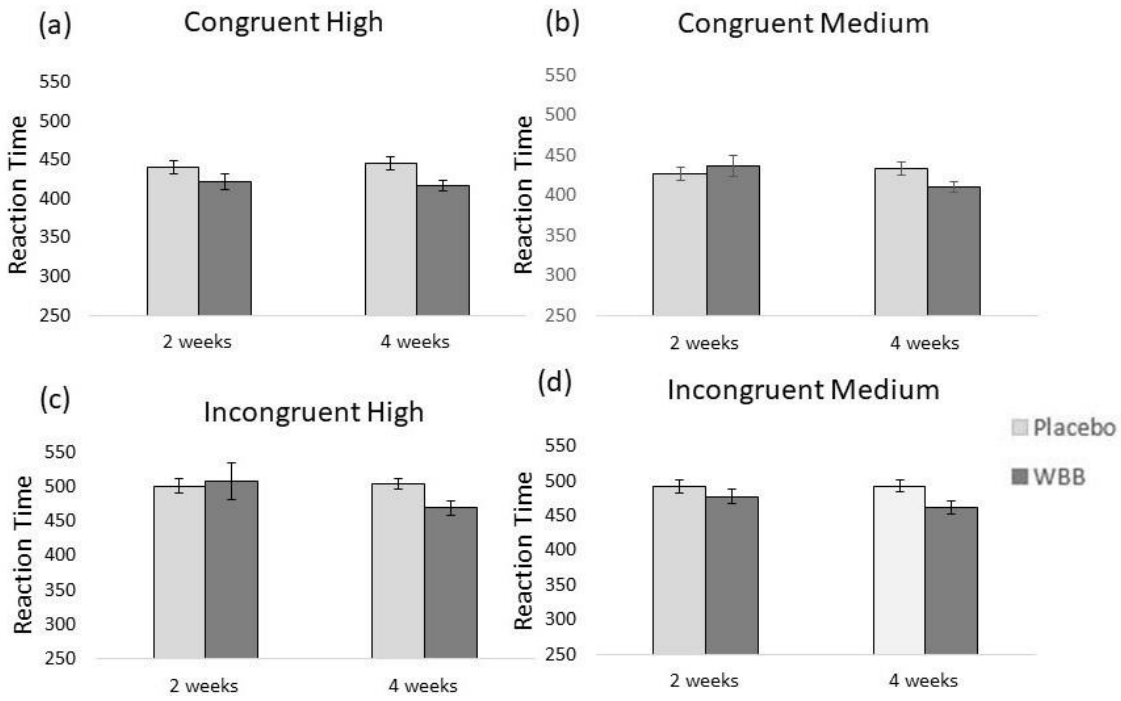

Figure 4. Mean (± standard error of mean) reaction time on MANT 2- and 4-weeks post-consumption of placebo and intervention drinks in adolescents aged 11-17 years: (a) Mean reaction time when congruent/high stimuli were presented. (b) Mean reaction time when congruent/medium stimuli were presented. (c) Mean reaction time when incongruent/high stimuli were presented. (d) Mean reaction time when incongruent/medium stimuli were presented.

Multiple linear regression analysis was conducted to examine the relationship between age, cognitive functioning and depressive symptoms assessed using MFQ. There was a positive significant correlation between MFQ scores and Rumination scores, indicating that those with higher rumination scores tend to report elevated symptoms of depression. The multiple linear regression model produced $F(7,60)=9.769$ $p<0.005$ with 50.6% of the variance explained by cognition including accuracy, reaction time, working memory and rumination. The standard coefficient B suggests that rumination was the only significant and the most influential predictor of the MFQ scores. None of the other predictors, including age, was a significant influence on MFQ score, with working memory assessed by KTT with the least influence.

10.4 Discussion

This randomised, placebo controlled, double blinded clinical trial investigated the chronic effect (2 and 4 weeks) of the wild blueberry drink on executive functioning, working memory and rumination in 11 to 17-year-old adolescents. There was no significant improvement in cognition due to chronic wild blueberry flavonoid intervention. Response interference, though

not significant, was evident and as expected, participants performed worse when more cognitively demanding incongruent high load trials were presented. After 4-weeks of flavonoid supplementation, there was no significant improvement in executive functioning, as assessed by accuracy and response time, or in working memory, assessed using the KTT.

There are many factors that may explain the lack of effect seen here, some of which include the sensitivity of the tasks for this age group, the length of the intervention, the source and dose of the flavonoid intervention, and the population targeted. For example, a 12-week chronic study using berry juice (grape and blueberry) reported improved verbal memory acquisition in older adults with cognitive difficulties [12,13]. Similarly, polyphenol supplementation of 5 weeks and 3 months was associated with faster spatial working memory in older adults [14,15]. On the other hand, there are several studies that failed to show any flavonoids related improvements in cognition. A 6-week cranberry juice and cocoa intervention study in older adults showed no significant benefits of the intervention on working memory [16,17]. Although comparison to existing literation is difficult due to the methodological inconsistencies between the studies.

Effects of wild blueberry flavonoids on rumination was also investigated. Rumination scores were lower for those who were allocated the flavonoid intervention; however, the lack of significance may be attributed to the fact that those with low mood were a small percentage of the sample recruited. This means that baseline rumination was not significantly elevated and that the effect size of any reduction would not be significant in a sample of this size i.e. the study was underpowered. Rumination is commonly present in those with depression and is believed to interfere with functioning, and treatment efficacy. It is also thought to impair concentration and memory by redirecting attention to personal concerns or irrelevant information [18]. It may result in a general deficit in the ability to switch from unhelpful strategies to helpful ones whilst performing a task. Rumination may also be associated with biases in information processing, where one has a tendency to specifically attend to and remember negative information rather than positive ones. Therefore, difficulty in inhibiting negative information and maintaining attention to positive distractors might be more evident in those who are depressed and ruminating. Rumination is therefore a relevant target for intervention. To our knowledge, no study has explored the effects of wild blueberry flavonoids on rumination. This study showed a significant association between rumination and depressive symptoms, hence any flavonoid related decrease in rumination scores may be a plausible mechanism by which flavonoids alleviates symptoms of depression. However, due to lack of depressed participants in this study, further studies in depressed population are

required to investigate the effects of flavonoids on rumination and the mechanisms by which these benefits, if any, are being mediated.

10.5 Conclusion

The analysis presented in this addendum did not show any significant influence of 4-week wild blueberry flavonoids on executive function, working memory or rumination, in a community sample of adolescents. As most of the existing studies have recruited either children or older adults, including those with cognitive difficulties, it is possible that the cognitive tasks used were not demanding enough to observe any effects of the flavonoid intervention. Additionally, a cognitive task battery containing measures of a wider range of cognitive domains would help determine the domain sensitive to flavonoid supplementation in this age group. The results cannot be generalized to a clinical population of adolescents suffering from depression, who may be exhibiting cognitive difficulties and find these measures more cognitively challenging.

10.6 References

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Chapter 11. Final Discussion

The overall aim of the five studies included in this thesis was to examine the effects of diet, specifically flavonoids, on depression in adolescents. This research builds on previous research investigating flavonoids effects on cognition in children and older adults. The first study systematically reviews and critically evaluates the existing literature investigating the association between nutrition and depression in children and adolescents. The four subsequent studies in this thesis focused on the use of dietary flavonoids and its association and influence on mood and cognition in young adults. This discussion will describe the findings from each study included in this thesis, compare these findings with the literature and explore the possible mechanistic aspects underlying the beneficial effects of wild blueberry flavonoid supplementation in young adults, evaluate the strength and limitations of the current research, and consider the implications for future research.

11.1 Overview of studies in the thesis

Study 1, the systematic review, critically evaluated previous research exploring the association between dietary intake of young adults and depression and related mental health problems. The lack of case control studies comparing nutritional profiles of those with elevated symptoms of depression to healthy young adults led to the rationale for the following study. Study 2 investigated the difference in the overall nutritional profile of young adults that were healthy and those with elevated symptoms of depression. These profiles were also compared to the daily recommended amounts of these nutrients as suggested by Public Health England and World Health Organisation. This provided a rationale to further explore the effects of flavonoid supplementation on mood and cognition in young adults. Effects of acute flavonoid intervention on transient mood and cognition in a community sample of young adults was then investigated in Study 3. Study 4 also investigated the effects of wild blueberry flavonoids on transient mood and cognition in a community sample of young adults. However, participants were screened for existing levels of depression and divided in to healthy and low mood groups to explore whether flavonoid supplementation is beneficial in these healthy and vulnerable young adults. Study 5 then explored the effects of 4-week chronic blueberry intervention on transient, sustained mood and cognition in young adults. These series of studies help further the existing knowledge on the benefits of dietary flavonoids on the health and wellbeing in this population. Additionally, it demonstrates the potential of flavonoids as a possible prevention and treatment strategy for depression in young adults.

11.2 Summary of Findings

11.2.1 Study 1: Is there an association between diet and depression in children and adolescents? A systematic review.

In the past decade, several studies have proposed that nutrition could play an important role in prevention and treatment of depression. The two approaches used to investigate this relationship consisted of exploring impact of individual nutrients or whole diet and eating patterns on mood. The literature primarily consists of studies reviewing the impact of individual nutrients for example omega-3 fatty acids, vitamins such as B12 and minerals such as zinc, selenium, and iron. However, studies systematically reviewing literature regarding overall diet and mental health especially in young adults is limited. Therefore, this study aimed to systematically review identified and evaluated research examining the relationship between diet and mental health in children and adolescents. A literature search was conducted using electronic databases such as PSYCINFO, MEDLINE, PUBMED and COCHRANE. Twenty studies were identified that met the inclusion criteria and were subsequently rated for quality. Methodological quality was assessed using National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. Effect sizes across studies were also calculated so that data could be compared on a single metric. Several inconsistencies between the studies were identified. These included methodological inconsistencies where a wide range of measures to assess dietary intake and mental health were used, some inappropriate for the age range of the sample, and inconsistencies in the use of key constructs where a range of different ways of defining and conceptualising diet quality were used which made comparisons between studies difficult. Despite the inconsistencies and some contradictory results, there was an overall evidence suggesting an association between consumption of a high-quality diet and lower levels of depressive symptoms. Likewise, there was a relationship between consumption of low-quality diet/unhealthy diet and depression/ poor mental health. However, where significant relationships were reported, effect sizes were small.

11.2.2 Study 2: Association between diet quality and depressive symptoms in young adults: A Pilot Study.

Several correlational studies exist reporting the association between individual nutrients and their abundance, or lack of, with symptoms of depression [(1)]. However, only a few studies have investigated the association between overall diet quality and depressive symptoms, especially in young people. Thus, this pilot study investigated this association by using the

EPIC Food frequency Questionnaire to calculate the diet quality using Diet Quality Index-International, in seventy-seven 11-18-years old healthy young adults, with the symptoms of depression measured using the Mood and Feelings Questionnaire (MFQ). Diet quality consisted of four components, adequacy, variety, moderation, and overall balance.

Though we did not find any significant association between diet quality and depressive symptoms, the amount of fruits and vegetables consumed were significantly lower than the daily recommended amount. Young people also consumed higher amounts of food containing empty calories than the amount recommended. Additionally, none of the participants achieved the suggested ratio of carbohydrate to protein to fat intake. Previous evidence strongly suggests a correlation between the amount of nutrients consumed and symptoms of depression. It is, therefore, also important to investigate the association between depressive symptoms and diet that did not meet the recommended level of nutritional intake. The lack of fruits and vegetables consumption and the related nutrients, even in healthy young adults, may potentially be one of the factors that contribute to the development of depression. Additionally, there is a possibility of a difference in the number of other micronutrients present in fruits and vegetables that were not assessed in this study. For example, flavonoids, present in abundance in brightly coloured fruits and vegetables, have shown to have a myriad of health benefits, including an improvement in cognition, which is associated with depression.

11.2.3 Study 3: Effect of Acute Blueberry Flavonoids on Mood in Children and Young Adults.

Given the lack of fruits and vegetables consumption in young adults and the limited research on the effects of flavonoids on mood, this study explored the acute effects of wild blueberry - flavonoids on cognition and transient mood in children (7-10 year olds) and healthy young adults (18-25 year olds). The adult participants were given both the intervention and the placebo treatment, the order of which was randomly assigned. Participants' transient mood and executive functioning was assessed pre and post intervention. Transient mood, as assessed by Positive and Negative Affect Schedule (PANAS), only revealed beneficial effect of flavonoid intervention on positive affect. The participants reported an increase in their positive affect after the consumption of wild blueberry drink but not the matched placebo. There was no noticeable difference in negative affect after the consumption of either treatment. Similar effects were seen in children in a crossover part of this study where they were randomly assigned to receive either the blueberry flavonoid intervention or the matched placebo. Further analysis of the subcategories of positive affect revealed that there was

significant increase in a subcategory Excitement 2 hours post blueberry intervention. This subcategory included a significant increase in participants' self-reported feelings of excitement, enthusiasm and inspiration. Mechanisms by which these mood states are being influenced remains unclear.

There was no significant effect of flavonoids on cognition assessed using Modified Attention Network Task (MANT). As expected, participants were less accurate and slower on more cognitively demanding trial, that is incongruent with higher load, making response interference evident. This, however, was not significant. Unlike previous studies flavonoid related improvement on participants' accuracy or response time was not observed. The lack of effects observed in this study may possibly be due to the methodological differences between the current and existing studies. Additionally, it's been speculated that flavonoids influence different cognitive domains in different age groups and the measures used may not have been sensitive enough to detect any changes in executive functioning in young adults [(2)].

11.2.4 Study 4: Effects of Acute Blueberry Flavonoids on Mood and Cognition in Healthy Young Adults with Depressive Symptoms.

In light of the recent evidence showing the beneficial effects of flavonoid consumption on mood in healthy children and young adults, this study aimed to investigate the effect of blueberry flavonoids on transient mood and cognition in healthy young adults and those with elevated symptoms of depression. Symptoms of depression was assessed by Patients Health Questionnaire (PHQ-9) and those who scored above the clinical cut off point were allocated to the low mood/at risk group. Transient mood was assessed using PANAS and further cognitive domains such as verbal fluency and working memory were assessed in addition to executive functioning, using Oral Word Association Task (COWATS), Keep Track Task (KTT), Serial 3's,7's and MANT. Participants were given both intervention and the matched placebo treatment, the order of which was randomly allocated. Mood and cognitive scores we recorded pre and post intervention. Contrary to previous findings, no flavonoid intervention related beneficial effects were observed in healthy participants or those with elevated symptoms of depression. There was an increase in positive affect after the consumption of wild blueberry intervention. However, this change was not significant and was only observed in healthy participants. Similarly, no significant improvement in accuracy, response time, verbal fluency and working memory was reported in both healthy and low mood groups due to the intervention. There was an improvement in the verbal fluency and the working memory post blueberry drink consumption. The change was more evident, though not significant in those with self-reported low mood. Again, response interference was evident, though not

significant, for both the groups where participants performed better on less cognitively demanding trials.

11.2.5 Study 5: Effect of 4-week Daily Wild Blueberry Supplementation on Depression in Adolescence.

Considering the evidence of acute flavonoid consumption on mood and cognition in the literature and the previous study in this thesis, this randomised, placebo-controlled, double-blinded trial investigated the effects of chronic blueberry flavonoid supplementation on symptoms of depression and its influence on cognition in young adults (12-18 year-olds). Unlike the previous studies, this study did not only measure transient mood but also assessed sustained mood (i.e. depressive symptoms). Any inferences made on sustained mood using the tools previously used are inappropriate as they only truly measure fleeting emotional states. On the other hand, using a validated screening tool for depression that assesses the affective and cognitive component of depression as used in this study helps to draw more accurate inferences about a global domain like depression in which the state (example, feelings of worthlessness) belongs to. Transient mood was assessed using PANAS, sustained mood using MFQ, executive function measured using MANT, working memory using KTT and rumination using Rumination Response Scale. Participants were allocated randomly to either the blueberry intervention or to the matched placebo drink. Their transient mood and cognition were assessed at baseline, 2 and 4 weeks post-intervention. Participants' sustained mood and rumination, however, were only assessed at baseline and 4 weeks post intervention. No beneficial effects of wild blueberry flavonoid were observed on transient mood. There was no significant improvement in positive effect 2 and 4weeks post-intervention nor was there a decrease in the negative affect after the chronic intervention. However, the results demonstrated that after 4 weeks of daily wild blueberry intervention there was a between-group difference in self-reported symptoms of depression. Those who were allocated to the wild blueberry intervention reported a decrease in depressive symptoms compared to those who were assigned the placebo drink. Participants' habitual fruit and vegetable intake was also measured using a food frequency questionnaire and it was found that participles' daily consumption of fruits and vegetables was significantly below the recommended amount as suggested by World Health Organisation. This was consistent with the results found in Study 1 and with other reports that suggest that only 18% of young people in the UK meet the daily requirement of fruits and vegetables. These results suggest that chronic supplementation with blueberry flavonoids may be beneficial in reducing depressive symptoms in a community

sample of young adults especially when they do not meet the daily recommended amount of fruits and vegetables.

Consistent with the previous studies, no improvement on cognition was observed due to chronic supplementation of wild blueberry flavonoids. There was no significant difference in participants' accuracy and response time at week 2 and week 4 when the treatment group was compared to the placebo group. Again, response interference was evident but not significant where participants in both groups performed worse on more cognitively demanding trials. Similarly, no significant beneficial effects of the chronic intervention were observed on working memory 2 and 4 weeks post daily supplementation. There was no significant difference in the rumination scores of those who received the wild blueberry drink and those who were assigned to the placebo drink. However, rumination scores of participants allocated to the wild blueberry drink were lower compared to those who received the placebo.

11.3 Discussion:

11.3.1 Mood effects: previous literature on acute mood changes following flavonoid-rich interventions are scarce and those on chronic mood changes even further limited. The beneficial effects of flavonoids on positive affect and depressive symptoms in the studies reported in this thesis are broadly in agreement with previously published data. Scholey et al. (2010) reported significant reduction in self-reported mental fatigue 2.5hrs post consumption of cocoa flavonoid [(3)]. Similarly, Boolani et al (2017) observed a reduction in anxiety following the consumption of cocoa, in young adults [(4)]. Massee et al (2015) demonstrated acute (2hr) benefits of cocoa supplementation on mental fatigue but no beneficial effects of chronic (30 days) supplementation [(5)]. Another study using blackcurrant juice as their flavonoid source used EEG data to highlight the anxiolytic effects caused by the intervention [(6)]. Additionally, a study investigating the effect of grape juice on mood in healthy adults showed that transient mood improved in those who received the flavonoid intervention [(7)]. The only other study that investigated chronic (8wks) effect using flavanone rich orange juice, reported no significant changes on positive affect in older adults [(8)]. On the other hand, there are a few studies that investigated the acute effects of flavonoid from different sources such as apples, cocoa and grape juice in healthy adults and showed no effect of the intervention on mood [(9-12)].

These inconsistencies in the literature may be due to the wide range of sources, doses and delivery methods of flavonoids used in addition to the difference in age groups across the studies. Another plausible reason for these discrepancies may be the differences in the

measures used to assess mood. Previous studies used tools that measure current emotional state rather that sustained mood or depressive symptoms. The more reliable measures such as PANAS and Bond Lader, used only by few, assess a specific narrowly defined states such as happy, sad, alert, or drowsy. However, sustained mood states are broad dimensions that are theorized to underline a global domain. For example, depression as a global domain contains more than just a few core emotions, which may be missed in the measures assessing narrowly defined states [13,14]. In this thesis, in addition to using a reliable measure of transient affect (PANAS), a reliable measure for sustained mood in adolescents (MFQ) was also included. The clinical trial in this thesis is the first to our knowledge to use an appropriate measure to assess depression and demonstrate the effects of chronic flavonoid intervention on depression symptoms. The cognitive components of depression and anxiety helps to draw more accurate inferences about a global domain (depression) in which the state (e.g. feeling of worthlessness) belongs to.

11.3.2 Cognitive effects: the studies in this thesis consistently showed no beneficial effect of wild blueberries on cognitive functioning measured by assessing participants' reaction time, accuracy, verbal fluency and working memory. It is difficult to compare these findings with previous literature due to the differences in cognitive tests used that covers broad range of cognitive domains. There is little consistency in regard to the test used within each domain. In addition to this the wide range of food products used as a score of flavonoids may also contribute to the inconsistencies in the literature. For example, a few studies have demonstrated significant improvements in working memory, measured using serial 3s and 7s, following acute doses of cocoa (1 to 2 hrs post) and ginkgo (6hrs post) in young adults [3,5,15]. Flavonoid profiles of cocoa and ginkgo are relatively different from each other and that of blueberry, making direct dose comparison difficult. Additionally, the absorption and metabolism rates will also vary reflecting the different flavonoid subclasses present in the dietary intervention used [16]. Another factor that may contribute to the inconsistencies in the results from the studies in thesis and previous literature is the age group of the participants recruited. A recent study using blueberry powder and purified extract in older adults showed intervention related improvements in episodic memories but not in working memory or executive functioning [17]. Further, a review found that flavonoid intervention in older adults resulted in improvements in episodic memory whereas executive function benefits may be common in younger adults [16]. A few other studies have shown beneficial effects of flavonoids in children on memory and attentional aspect of executive function [18-21]. Study 5, in this thesis, is the first to our knowledge to explore the effects of flavonoids on cognition in adolescents. As majority of the tests used were previously used in children or older adults,

they may not have been sensitive enough to measure any changes that may have occurred due to the intervention.

Study 5 is also the first to our knowledge to explore the effects of chronic wild blueberry intervention on rumination. It is a pattern of recursive thinking focused on one's negative mood and involves a broad range of cognitive and affective sub processes that are associated with activation in a wide region of the brain [22]. These include attention, self-reflection processing and recall of autobiographical memories. It is suggested that increased tendency to ruminate exacerbates the neural processing of negative information not only in depressed individual but also in those who are healthy [21]. Therefore, we hypothesised flavonoid related benefits on rumination may be an underlying mechanism by which participants experience elevated mood. However, we found no significant intervention related changes in self-reported rumination. This requires replication in larger group of depressed individuals and over several time points.

Overall, this thesis provides evidence that consumption of blueberry flavonoids has potential to positively affect transient and chronic mood in young people. Due to the wide range of flavonoid sources, doses, delivery methods, intervention duration and cognitive measures used across the studies, it is difficult to compare these results with previous literature. The outcome measure in terms of mood, i.e. transient or sustained, should be taken into account and a more systematic approach is required to explore the specific domains of cognitive function influenced by flavonoid administration, and to further explore how these altered cognitive functions may influence mood, for example addressing the omission of cognitive processes such as rumination whilst exploring the effects of flavonoids on mood.

11.4 Possible mechanistic aspects underlying the beneficial effects of flavonoid supplementation

Flavonoids are known to have diverse pharmacological effects with cell and animal studies reporting its antidepressant-like effects [23,24]. Human studies investigating the mechanisms by which these antidepressant-like effects are mediated are limited and requires greater consideration. Several possible mechanisms are hypothesised to be the underlying cause of these antidepressant-like effects.

11.4.1 Influences of flavonoids on cerebral blood flow: Anthocyanin interventions increase dilation mediated blood flow and cerebral blood flow [25,26]. Increased cerebral blood flow to areas such as dorsolateral prefrontal cortex (DLPFC), a site within the frontal lobes highly associated with cognitive control [27] and emotional regulation [28], may strengthen neural

circuitry in this area. Although the studies reported in this thesis did not find any effects of flavonoids on executive functioning or rumination, previous research has shown an improvement in executive functioning and that is consistent with the evidence linking executive functioning and low mood and suggests an indirect pathway whereby flavonoid consumption enhances cerebral blood flow, boosting executive functioning, and thus helping to inhibit cognitive features (i.e., rumination) that maintain depression.

11.4.2 Influences of flavonoids on Neurotransmitters: Most flavonoids are able to have an effect on monoamine neurotransmitters (i.e. 5-HT, Noradrenaline, Dopamine) that are involved in mood regulation, in the brainstem and hypophysis cerebri [29,30]. Flavonoids result in an increase in the bioamine content by restricting the bioamine reuptakes by the synapses and restricting the Monoamine Oxidase (MAO) activities. MAO is involved in oxidation of the monoamine neurotransmitters. Research has shown that some flavonoids have similar structure to the synthetic MAO inhibitors. It, therefore, blocks MAO activities to raise the content of serotonin (5-HT), noradrenaline and dopamine in the neuronal synapses alleviating the symptoms of depression [32,31]. Another way by which flavonoids are hypothesised to elevate mood is by limiting the number of serotonin receptors to prevent serotonin reabsorption and inhibiting the activity of catechinic acid-O-trans methylase. This in turn results in an increased expression of brain monoamine neurotransmission [32,35].

11.4.3 Influences of flavonoids on neuroendocrine system: Animal studies have shown that chronically stressed rats when given flavonoid supplementation effected the Hypothalamic-pituitary-adrenal (HPA) axis. It inhibited the stress hormones levels and the upregulation of hippocampal glucocorticoid receptors expression. It was also shown to prevent nerve cell damage caused by high corticosterone concentrations, that are also known to bind to 5-HT [36,37]. Additionally, many flavonoids are inhibitors of triphosadenine and acetycholine. Some may restrain ATP and α-amino-3-OH-5-methane acid, thereby interacting with 5-TH receptors, α1 and α2-adrenoceptors, resulting in antidepressant activity [38].

11.4.4 Flavonoids anxiolytic effect: The ability of flavonoids to mimic anxiolytic-like effects by binding to benzodiazepine receptors which influences the effects of Gamma Amino Butyric Acid (GABA) via GABAA receptors [34,39] may be another possible way it influences mood. GABA is an inhibitory neurotransmitter present exclusively in the central nervous system. In addition to regulating cognition, decreased levels of GABA are associated with mood disorders. However, the absence of an effect of flavonoid consumption on negative affect (a strong indicator of anxiety) in the studies in this thesis suggests that the GABA hypothesis may not be a plausible explanation for the observed acute effects on positive

affect. However, the chronic effects of flavonoid on sustained mood may be the result of flavonoids' anxiolytic effects.

11.4.5 Conclusion: In the light of the results from the studies in this thesis, flavonoids inhibitory effects on MAO is the most plausible mechanism by which transient and chronic mood is being affected. As in Study 3, PA scores significantly improved with no changes in NA post WBB intervention. This suggests an improvement in depressive symptoms, but not anxiety. Similarly Study 5 demonstrated improvements in MFQ scores (depressive symptoms) but not RCADS (anxiety symptoms) post WBB intervention. Further, no effect of WBB intervention was demonstrated on cognition, including reaction time, attention, working memory, verbal fluency, and rumination. Overall, the thesis results suggest that improvement in mood via improvement in cognition, or via anxiolytic effects, did not explain the improvement in transient and chronic mood in young adults.

11.5 Limitations and further work

One of the biggest limitations of this thesis is the relatively small number of participants with elevated symptom of depression that were recruited in studies 2, 4 and 5. This made it difficult to compare the healthy and low mood group and limited statistical power. In Study 3 we did not distinguish between healthy and low mood participants as participants were not screened for existing symptoms of depression at baseline. For practical reasons, self-report questionnaires of depression symptom severity can be used and we included these in studies 2 to 5. However, ideally, our understanding of mechanisms linking flavonoids and depression would be maximised if we were able to recruit participants who had a diagnosis of depression confirmed by standardised clinical interviews.

The food frequency questionnaire used in Study 2 was modified by including pictures of portion sizes to help participants estimate their food intake. It was still, however, a self-reported measure. Participants report approximate food intake which is then converted into energy and nutrient intake. Nevertheless, the amount of energy and nutrients derived from the food intakes may not be the amount available to the individual for metabolism. These measurements therefore only provide an estimate of the nutrients that are available to the individual. Additionally, quantitative measurement of dietary intake can only be made over a short period of time and therefore cannot capture fluctuations in food intake.

A more accurate and reliable method of measuring energy and nutritional intake would be by chemical or other methods of analysing the energy nutrient content of the food and beverages that have been consumed by an individual. Measures that are biochemical such as a

physiological marker that reflects energy or nutritional intake would provide a more accurate nutritional profile of not only what is consumed, but also what has been available for metabolism and the amount absorbed by the digestive system. Biochemical measures used for this purpose include energy expenditure, urinary breakdown of proteins, sodium and potassium, plasma levels for vitamins, and tissue levels of minerals and fatty acids. Similarly, for the intervention studies, flavonoid levels measured using a biochemical method at baseline and post intervention would help to accurately analyse the average amount of flavonoids consumed habitually and the amount absorbed from the wild blueberry drink. This would also be a more reliable way to assess participant adherence to the low flavonoid diet they were instructed to follow 24 hours prior to the session and to the daily flavonoid drink. Another limitation of the intervention studies was the fact that no record of the breakfast or food consumed 24 hours prior was obtained. There is a possibility that any beneficial effect of flavonoids on negative affect may have been masked by other macronutrients because of the inconsistency in the type of breakfast participant may have consumed. Future studies should take this into account and provide a low flavonoid meal or to instruct participants to adhere to a certain diet plan.

The chronic study (Study 5) was also heavily reliant on participants and their parents' ability to adhere to the protocol. A biochemical measure of flavonoid levels of the participant would help determine if the protocol was adhered to as well as participants' ability to metabolise and absorb flavonoids. Absorption of dietary flavonoids depends on its biochemical properties such as molecular size configuration, lipophilicity and solubility. Dietary flavonoids are either absorbed from the small intestine or the colon depending on whether the structure of the flavonoid is glycoside or aglycone. Aglycon can easily be absorbed by the small intestine whereas flavonoid glycoside must be converted to aglycon form. Complications in this conversion or absorption through the small intestine or colon would result in failure to see any mood or cognitive improvements due to flavonoids.

In addition, a 4-week food diary would ensure that the effects observed was due to the intervention and that the daily food consumption was not a significant factor in the changes observed. Although, getting adolescents to agree keep a 4-week food diary with accuracy may prove difficult.

The cognitive battery used to assess the effect of flavonoids on various cognitive domains have not been demanding for this age group. The test battery used is sensitive to changes in cognition in children and older adults, especially those with cognitive difficulties. We did not

assess cognitive difficulties in adolescent participants and so it is possible that difficulties such as dyslexia may have obscured any effects.

Future studies should include a larger sample especially of those with self-reported symptoms of depression. A comparison of flavonoid effect on transient and sustained mood between community samples of young adults with a diagnosis of depression and those without depression would also be of value in determining the potential of flavonoids to prevent or even treat depression. Additionally, longitudinal studies in young adults that are at risk of developing depression would also be valuable in investigating flavonoids as a preventative strategy for depression in this population. Studies focused on investigating the biochemical changes and investigating the mechanistic pathways by which flavonoids seem to influence this decrease in depressive symptoms in humans is also of great importance.

11.6 Final Conclusion

Depression in young adults is common, severe and leads to immediate and long-term morbidity and mortality. The rate of underdiagnoses and undertreatment of depression in this population is increasing the global burden of this disease. Due to limited evidence-based treatment strategies, prevention strategies in groups that are higher at risk of developing depression is becoming increasingly important. The acute and chronic studies in this thesis support increasing evidence emerging from the literature suggesting a relationship between flavonoids and depression. These series of experiments are the first to consider the effects of a flavonoid rich intervention on depressive symptoms in young adults. These results, however, are preliminary and warrant further focused investigation on the association between flavonoid-rich interventions and mental health in young adults. There is a potential that depressive symptoms can be improved in healthy young adults due to flavonoid rich interventions indicating a potential preventative strategy for depression especially those who are at risk of developing this disorder and experiencing distress but not meeting the criteria for treatment.

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12.4 Appendix 4: Published Version of Study 3

nutrients

MDPI

Article

Effects of Acute Blueberry Flavonoids on Mood in Children and Young Adults

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Abstract: Epidemiological evidence suggests that consumption of flavonoids (usually via fruits and vegetables) is associated with decreased risk of developing depression. One plausible explanation for this association is the well-documented beneficial effects of flavonoids on executive function (EF). Impaired EF is linked to cognitive processes (e.g., rumination) that maintain depression and low mood; therefore, improved EF may reduce depressionogenic cognitive processes and improve mood. Study 1: 21 young adults (18–21 years old) consumed a flavonoid-rich blueberry drink and a matched placebo in a counterbalanced cross-over design. Study 2: 50 children (7–10 years old) were randomly assigned to a flavonoid-rich blueberry drink or a matched placebo. In both studies, participants and researchers were blind to the experimental condition, and mood was assessed using the Positive and Negative Affect Schedule before and 2 h after consumption of the drinks. In both studies, the blueberry intervention increased positive affect (significant drink by session interaction) but had no effect on negative affect. This observed effect of flavonoids on positive affect in two independent samples is of potential practical value in improving public health. If the effect of flavonoids on positive affect is replicated, further investigation will be needed to identify the mechanisms that link flavonoid interventions with improved positive mood.

Keywords: depression; mood; affect; dysphoria; cognition; flavonoid; blueberries; children; young adults

1. Introduction

Major depressive disorder is the leading international cause of disability and is estimated to affect 350 million people worldwide [1]. It is the second most common cause of death in 15–29 years old, via suicide [1]. Current treatments for depression include psychological therapies and a range of pharmacological agents. The treatment options recommended for children and adolescents are limited, with only one recommended pharmacological treatment, fluoxetine, in addition to psychotherapy. These treatment options are further constrained because of concerns about the use of anti-depressant medication with young people, and because most young people do not have easy access to psychological therapies [2–4]. Therefore, there is a pressing need for alternative interventions, especially those that offer a cost-effective and practical means of preventing, or alleviating, depression in this population.

A common symptom of depression is impaired cognitive functioning, with significant deficits in executive functioning (EF). EF is an umbrella term, describing cognitive processes such as working memory, planning, problem-solving, cognitive flexibility, inhibitory control, directing attention, thoughts and, therefore, behaviours. Impaired EF is believed to maintain depressive symptoms,

such as negative self-perception and low mood, via perseveration and rumination [5–8]. Importantly, EF is associated with the development of the frontal area of the brain, an area that continues to mature and develop throughout adolescence and into early adulthood [9,10]. Thus, any disturbance to the development of the frontal region during this critical period (for example because of an episode of depression) can have a long-lasting impact, and may explain why depression that occurs during adolescence and early adulthood is associated with long-term impairments into adult life [11,12].

Flavonoids are a class of polyphenols (micronutrients) found naturally in fruits, vegetables, tea, coffee and cocoa. Flavonoid consumption has been associated with both vascular and cognitive benefits across the lifespan [13–17]. Single-dose flavonoid interventions have produced improvements in attention, inhibition, visuospatial memory, and executive function between 2–6 h post-consumption [14,18–20], whilst supplementation of flavonoids for 1.5–8 weeks has been associated with improved visuospatial memory and improved long-term memory [15,21,22]. Numerous mechanisms of action have been investigated to explain the beneficial effects of flavonoids on cognition. These include increases in cerebral blood flow, protecting against neuronal stress via anti-inflammatory and anti-oxidative effects, and positively stimulating neural signaling pathways, such as Extracellular Signal-Regulated Kinase (ERK), Serine/Threonine-specific Protein Kinase (Akt) and Brain-Derived Neurotrophic Factor (BDNF), leading to improved neural signaling [15,21,23,24].

Independently of the experimental evidence that shows flavonoids improve cognitive performance, there is emerging evidence that flavonoids may also support mental health and well-being. Epidemiological data shows that lifetime consumption of fruit and vegetables (and therefore higher flavonoid consumption) predicts a lower incidence of depression in later life [25–29]. The benefits are also seen earlier in life; a recent systematic review concluded that whilst the quality of evidence was weak, there was a consistent body of research reporting cross-sectional and longitudinal associations between nutrition and mental health in children and young people [30]. Similar findings have been shown by other authors [31,32]. However, there is an absence of studies exploring the effects of flavonoid-rich interventions on mood.

Given the well-documented links between flavonoid consumption and cognition, and between cognition and depression, the studies reported in this paper assess the acute effects of flavonoid-rich wild blueberries (WBB) on mood two hours post-consumption. This two-hour interval coincides with the time-frame for the peak absorption and metabolism of the anthocyanins present in blueberries [33]. In addition, it is important to establish whether acute effects on mood are observable prior to considering a chronic flavonoid-based intervention for mood outcomes. Two independent groups, healthy children and young adults, were recruited. These groups represent individuals who are at crucial stages of mental and cognitive development and thus plausible points at which prevention and public health interventions may be particularly powerful.

2. Materials and Methods

The research was reviewed and given a favorable ethical opinion for conduct by the University of Reading Research Ethics Committee (2015-148-CW & UREC 15/10) and was conducted in accordance with the Declaration of Helsinki. All participants were screened for food related allergies or other health conditions, e.g., diabetes, heart disease, blood pressure, thyroid, kidney and liver diseases which would exclude them from the study.

2.1. Study 1 (Young Adults)

Participants: 21 undergraduate students were recruited from University of Reading; 19 females and two males aged between 18 and 21 years (Mean (M) = 20.14 years, Standard Deviation (SD) = 1.01).

Drink preparation and consumption: All interventions were prepared on site, no more than 20 min before consumption, by an independent researcher who did not administer the drink to participants. The flavonoid-rich wild blueberry (WBB) drink contained 253 mg anthocyanins and was prepared by mixing 30 g of freeze-dried WBB with 30 mL of low-flavonoid Rocks Orange Squash and 220 mL of

For the main test sessions, the PANAS-C was administered alongside a 40 min cognitive battery which included Rey's Auditory Verbal Learning Task (RAVLT), Modified Flanker Task (MFT) and Test of Word Reading Efficiency (TOWRE-2) [37–39]. The cognitive performance data is reported elsewhere [40].

PA and NA were calculated by summing positive and negative items using the validated children's version of the PANAS; PANAS-C [36]. The PANAS-C has 30 items (15 positive and 15 negative emotions) and includes the original 20 items from PANAS-NOW and 10 additional child-friendly synonym items derived from the PANAS-X (Expanded Form). This was administered and analysed as in study one.

Procedure: This study was also double-blind and placebo-controlled. A between-groups design was used to minimise disruption to the school and demand on participants. All children took part in a screening session one to two days before the main test day. The main test day consisted of a baseline session and a post-consumption session 2 h later. All parents or legal guardians gave written consent for their child to take part, and each child gave verbal assent before any research began. Parents confirmed that their child had no allergies or food intolerances that would prevent them from taking part. To accommodate school hours, children were tested during the afternoon at school. They were not required to fast prior to testing. However, parents were asked to make sure that their child consumed a low-flavonoid diet for 24 h before the baseline session, including breakfast and lunch on the main test day. Parents were telephoned after their child's screening session to give them the date of their child's next test day, and to remind them about the dietary restrictions. School canteen staff members were also asked to monitor children's meals on the day of testing and to remind the children taking part in the study not to eat high-flavonoid foods for lunch that day.

Children were tested individually in a quiet space at school on two separate occasions, one to two days apart. On day one, children completed the screening and practice tasks outlined previously. On testing day 2, after a low-flavonoid lunch, participants completed the PANAS-C and cognitive battery (data reported elsewhere [40]), before consuming the placebo or flavonoid drink. They then returned to their classrooms and were asked not to exercise or consume anything except water. After 2 h, participants individually completed the PANAS-C and a matched version of the cognitive battery again in the presence of the researcher. Participants were given a written debrief about the study aims and were given similar debrief information to take home to their parents.

2.3. Analysis

Data was analysed using SPSS (Version 22.0). In both studies, PA and NA were dependent variables in a two-way analysis of variance (ANOVA) with Drink (placebo, WBB) and Session (pre- and post-consumption) as independent variables. For study 1, this was a fully repeated measures 2 × 2 ANOVA (as participant consumed both drinks and were tested at all time points), and for study 2 this was a mixed 2 × 2 ANOVA (as participants consumed either the placebo or WBB, and were tested at both time points). Significant main effects and interactions were explored with Bonferroni corrected post-hoc t-tests. Baseline differences in intelligence quotient (IQ (study 2) were examined using one-way ANOVAs with Drink (placebo and WBB) as the independent variable.

3. Results

3.1. Study 1 (Young Adults)

Figure 1 shows PA and NA before and after consumption of the placebo and WBB drinks. There was no significant main effect of Drink on positive affect ($F(1,20) = 1.24$, $p = 0.28$). There was a significant main effect of Session ($F(1,20) = 10.67$, $p = 0.004$) and a significant Drink × Session interaction ($F(1,20) = 7.5$, $p = 0.013$). Subsequent Bonferroni-corrected post-hoc t-tests demonstrated that there was a significant increase in PA after consuming the WBB drink (pre: M = 22.76, SD = 9.01 and post: M = 29.48, SD = 8.60; $t(20) = -4.68$, $p < 0.001$). There was no change in PA after consuming

water. The placebo drink was matched to the WBB drink for vitamin C (4 mg), sugars (8.90 g fructose, 7.99 g glucose), 30 mL Rocks Orange Squash and 220 mL of water. Drinks were prepared in an opaque cup and straw to ensure that double-blinding was maintained.

Mood Measure: The Positive and Negative Affect Schedule-NOW (PANAS-NOW) was used to assess current mood. The PANAS-NOW is a valid and reliable 20-item (10 positive and 10 negative mood states) self-report measure of Positive Affect (PA) and Negative Affect (NA) [34,35] which can be used on multiple test occasions. Participants were asked to rate the degree to which they were currently experiencing each item, on a five-point Likert scale. The ratings of positive and negative items were summed to calculate an overall positive and overall negative affect score, ranging from 10–50 (lower scores indicating lower levels of positive or negative affect). A cognitive performance assessment (Modified Flanker Task; MFT) was also administered after each presentation of the PANAS, the data for which will be reported elsewhere.

Procedure: This was a double-blind, placebo-controlled, crossover study. Informed consent was obtained from all participants for inclusion before they participated in the study. To minimise variability caused by prior flavonoid consumption, participants were given a list of high polyphenol food items (such as tea, coffee, chocolates, most fruit and vegetables) and asked not to consume these for 24 h before each test session, including the morning of the sessions. All test sessions were scheduled in the morning and all participants attended three test sessions separated by a minimum three-day wash-out period (range three to seven days; median three days). This included a practice test day (Screening), which was described to the participants as the first test day, where all participants received the placebo drink. This was to ensure participants were fully versed in the experimental procedures before testing began. Thereafter they were tested on two further occasions, during which the placebo or flavonoid intervention was administered to each individual in a random order (10 received WBB first).

On each of the three visits to the laboratory, participants completed the baseline mood measure (PANAS-NOW) on arrival. Immediately following this they consumed the drink. They were asked to return to the laboratory 2 h later and refrain from eating, exercising or drinking during this time. However, water consumption was permitted before and during the 2 h break. Upon their return, participants completed the PANAS-NOW again. Overall, each visit to the laboratory lasted approximately 30 min.

2.2. *Study 2 (Children)*

Participants: 52 participants (29 female) aged seven to ten years old (Mean age = 8.241, SD = 0.965) were recruited from two local primary schools in Berkshire, UK.

Drink preparation and consumption: On the test day, participants were randomly allocated to receive the wild blueberry drink (n = 28; 17 females; Mean age = 8.236, SD = 0.869) or a matched placebo (n = 24; 12 females; Mean age = 8.227, SD = 1.031). The contents of each drink were identical to Study 1, but instead only included 170 mL of water to aid consumption and palatability in a child population. A confederate prepared all drinks at the school immediately before they were administered to the participants by the researcher. Drinks were offered in an opaque drinking cup with a black straw placed through the lid to ensure double blinding.

Measures: A computerised version of the children's Raven's Coloured Progressive Matrices (RCPM) was administered at screening to measure fluid intelligence. This is a well validated measure assessing non-verbal and reasoning abilities [36]. This measure was included to ensure all participants were of healthy cognitive functioning for their age, and to ascertain intervention effects in relation to intelligence. The York Assessment of Reading Comprehension was administered to ascertain whether participants were at an age-appropriate reading comprehension level. A modified Continuous Performance Task was also employed to assess possible attentional deficits. Data from these three tasks were not used in the analysis of the current study. A practice of the child version of the Positive and Negative Affect Scale (PANAS-C) and cognitive task battery was also undertaken to ensure understanding of the tasks and reduce practice effects. This practice data was not analysed.

the placebo drink (pre: M = 24.0, SD = 9.59 and post: M = 25.24, SD = 8.50; $t(20) = -0.73$, $p = 0.48$, Figure 1a). Post-hoc tests showed no significance difference in PA and NA between drinks at baseline ($p > 0.05$) but there was a significant difference in PA scores post-consumption between the placebo and WBB drink ($t(20) = 2.286$, $p = 0.033$). The main effect of Session was explained by an increase in PA post-consumption (M = 27.36, SD = 8.55) relative to pre-consumption (M = 23.38, SD = 9.3), which, as indicated by the significant interaction, was driven by the WBB drink.

There was a significant main effect of Session on NA ($F(1,20) = 8.30$, $p = 0.009$). After consuming both placebo and WBB drinks, the participants reported a reduction in NA (pre: M = 13.17, SD = 2.67 and post: M = 12.19, SD = 2.49; Figure 1b). This may be explained by postprandial blood glucose effects due to the matched sugar content of both drinks. There was no significant main effect of Drink on NA ($F(1,20) = 0.67$, $p = 0.42$) and no significant Drink x Session interaction on NA ($F(1,20) = 0.51$, $p = 0.49$).

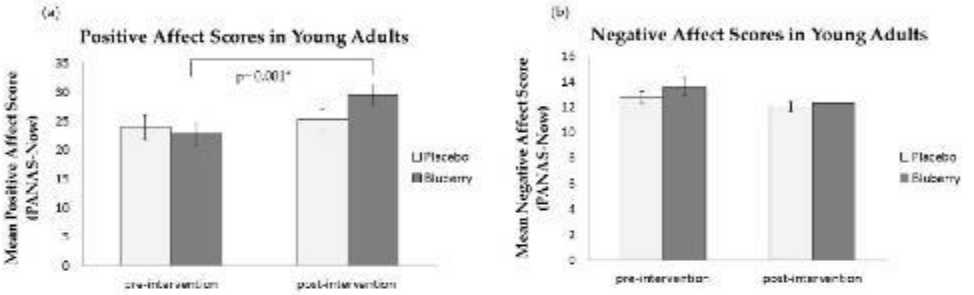

Figure 1. Mean PANAS-NOW Mood scores in adults aged 18–21 years: (**a**) Mean PA scores pre- and post-consumption of placebo and WBB drinks; (**b**) Mean NA scores pre- and post-consumption of placebo and WBB drinks.

3.2. Study 2 (Children)

Forty-nine participants were included in the analysis of IQ due to three missing data files. There were no significant differences at baseline between groups for IQ (Ravens: WBB group, M = 26.78, SD = 4.31; Placebo group, M = 26.55, SD = 5. 98; $F(1,47) = 0.024$, $p = 0.878$), PA (WBB group, M = 49.00, SD = 11.96; Placebo group, M = 49.21, SD = 10.04; $F(1,50) = 0.005$, $p = 0.947$) or NA (WBB group, M = 20.79, SD = 7.43; Placebo group, M = 19.25, SD = 3.86; $F(1,50) = 0.832$, $p = 0.366$).

Figure 2 shows PA and NA in both groups before and after the intervention. There was no significant main effect of Session ($F(1,50) = 1.362$, $p = 0.249$) or Drink ($F(1,50) = 0.456$, $p = 0.503$) for PA. However, there was a significant Session × Drink interaction ($F(1,50) = 4.176$, $p = 0.046$). Paired samples *t*-tests for each condition revealed no significant change in PA after consuming the placebo drink (pre: M = 49.21, SD = 16.04 and post: M = 48.29, SD = 15.51; $t(23) = 0.564$, $p = 0.578$), but a significant increase in PA after consuming the WBB drink (pre: M = 49.0, SD = 14.86 and post: M = 52.36, SD = 14.36; $t(27) = -2.495$, $p = 0.019$; Figure 2a). There was no significant difference in PA between placebo and WBB at the post-consumption time point ($t(52) = -1.597$, $p = 0.116$).

Negative Affect in both groups is shown in Figure 2b. There was no significant main effect of Session ($F(1,50) = 0.009$, $p = 0.927$) or Drink ($F(1,50) = 0.355$, $p = 0.554$) on NA. There was also no significant Session × Drink interaction ($F(1,50) = 0.453$, $p = 0.504$; Figure 2b). NA did not change 2 h after consuming either the placebo (pre: M = 19.25, SD = 8.73 and post: M = 19.79, SD = 9.78) or the WBB drink (pre: M = 20.79, SD = 8.09 and post: M = 20.07, SD = 9.06).

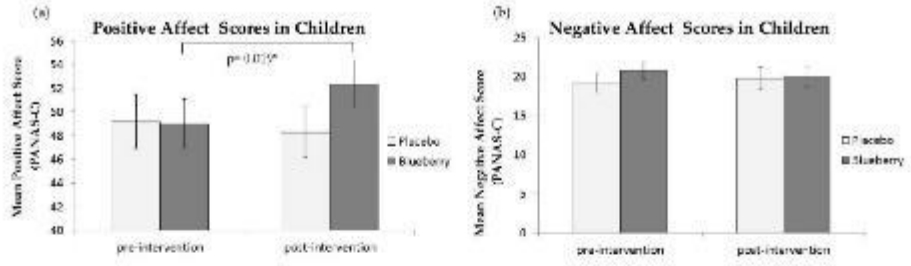

Figure 2. Mean PANAS-C scores in children aged 7–10 years: (**a**) Mean PA scores pre- and post-consumption of placebo and WBB drinks; (**b**) Mean NA scores pre- and post-consumption of placebo and WBB drinks. * Significant at <0.05. Attained from post-hoc paired samples *t*-test.

4. Discussion

These randomised, placebo-controlled, double-blind studies investigated the effects of acute consumption of a flavonoid-rich wild blueberry drink on the mood of healthy children and young adults. In both studies, increased Positive Affect was observed 2 h after consumption of the flavonoid-rich WBB drink (significant drink by session interaction). The flavonoid drink had no effect on Negative Affect. The effect of flavonoids on mood was consistent across two populations, at two different time points (morning and afternoon), and in a between- and within-subject design. Thus, the positive effect of blueberry flavonoids on Positive Affect appears to be robust to variations in experimental design.

The distinctive effect of flavonoids on PA but not NA is notable. PA and NA reflect orthogonal facets of mood. A low PA is more highly linked to depression, and high NA is more closely related to anxiety [34–36]. Thus, these data suggest that the effect of flavonoid consumption on mood may be specific to depressive disorders, rather than pervasive across different mood states. In the young adult study, both drinks led to a decrease in NA. This may be due to the sugar content of both the drinks, as dietary carbohydrates have been shown to enhance the uptake of circulating tryptophan (a precursor of serotonin) into the brain by promoting insulin secretion [41,42].

Although preliminary, these results are intriguing and warrant focused investigation of the relationship between flavonoids and mood, as well as with mental health more generally. It is important to note that diagnosis of mental health disorders or consumption of medication were not specific exclusion criteria; however, the data showed normal levels of PA and NA [34,43], indicating a healthy sample. The young adult participants were predominantly female, and therefore these results may not be generalisable to a male population; however, there is no evidence to suggest a gender-specific mechanism underlying the effects of flavonoids on the brain. The child sample was of average IQ, and the young adult population was recruited from a University population and thus likely to have an average to above-average IQ. No carry-over effects of flavonoids are expected as the half-life of flavonoids is estimated to range from 2–28 h and there was a minimum of three days insured between the test days [44].

Mood is by definition a short-term experience. In non-clinical populations mood is usually labile. However, sustained periods of low mood (dysphoria) are a strong predictor of the emergence of major depressive disorder. Therefore, if acute flavonoid consumption improves Positive Affect, sustained consumption of flavonoids may help prevent dysphoria, and thus, major depression. Given that depression tends to emerge for the first time during adolescence or early adulthood, and is likely to reemerge as a relapse later in life, an intervention which increases flavonoid consumption during this critical period of development could decrease the incidence of adolescent and life-long depression.

The mechanism linking flavonoids and mood is not known and requires greater consideration. There are a number of plausible mechanisms which may explain these results. First is the finding that flavonoids increase cerebral blood flow [45]. One of the last brain structures to mature is the

dorsolateral prefrontal cortex (DLPFC), a site within the frontal lobes highly associated with cognitive control [46] and emotional regulation [47]. Increased cerebral blood flow to this area may help strengthen neural circuitry in the frontal lobes, where cognitive and emotional control is located. This is consistent with the evidence linking executive functioning with low mood, and suggests an indirect pathway whereby flavonoid consumption enhances cerebral blood flow, boosting executive functioning, and thus helping to inhibit cognitive features (i.e., rumination) that maintain depression.

A second, alternative explanation for the link between flavonoids and mood is the effect of anthocyanins (a subtype of flavonoids) on Monoamine Oxidase (MAO) inhibition. MAO is involved in the oxidation of monoamines, some of which are neurotransmitters involved in the regulation of mood (e.g., serotonin, dopamine, and noradrenaline). MAO inhibitors have been used to treat mood disorders. Thus, the consumption of fruits high in flavonoids, such as blackcurrants, may significantly reduce MAO activity, thereby increasing circulating monoamines, and elevating mood [48]. This would suggest a direct pathway between flavonoid consumption and mood.

A third possible mechanism is the ability for flavonoids to mimic anxiolytic-like effects by binding to benzodiazepine receptors, which influences the effects of Gamma Amino Butyric Acid (GABA) via $GABA_A$ receptors [49,50]. GABA is an inhibitory neurotransmitter present exclusively in the central nervous system. In addition to regulating cognition, decreased levels of GABA are associated with mood disorders. However, in the two studies reported here, the absence of an effect of flavonoid consumption on NA (a strong indicator of anxiety) suggests that the GABA hypothesis may not be a plausible explanation for the observed acute effects on PA.

5. Conclusions

This study demonstrated acute effects of blueberry flavonoid consumption on Positive Affect and no effect on Negative Affect in healthy children and young adults. Dietary interventions could play a key role in promoting positive mood and are a possible way to prevent dysphoria and depression. Given the potential implications of these findings for preventing depression, a disabling and common mental health problem in adolescents and adults, it is important to replicate the study and assess the potential to translate these findings to practical, cost-effective and acceptable interventions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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12.6 Appendix 6: Patient Health Questionnaire -9

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

12.7 Appendix 7: Study 4 Mean Accuracy Scores Assessed using Modified Attention Network Task (MANT)

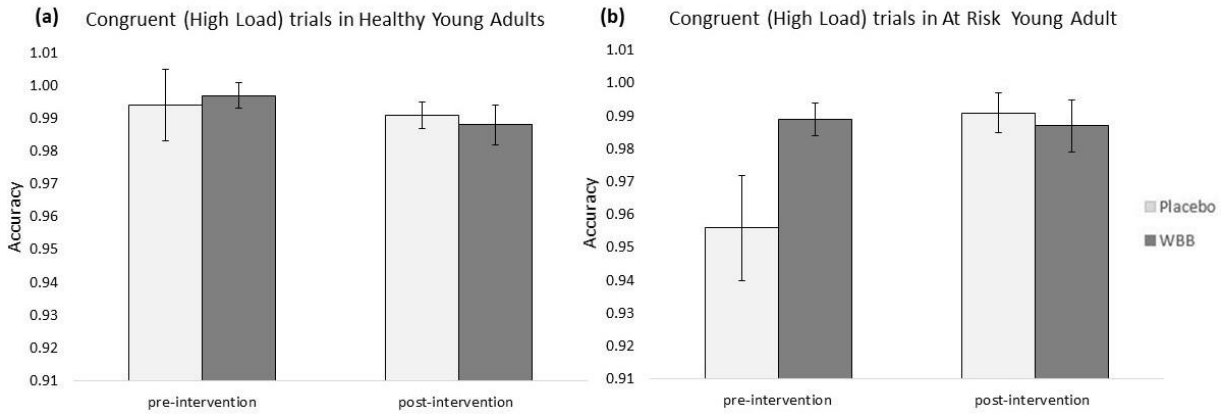

Figure 1. Mean (± standard error of the mean) Accuracy score on MANT in young adults aged 18-21 years: (a) Mean Accuracy score when congruent/high stimuli was presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Accuracy score when congruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression

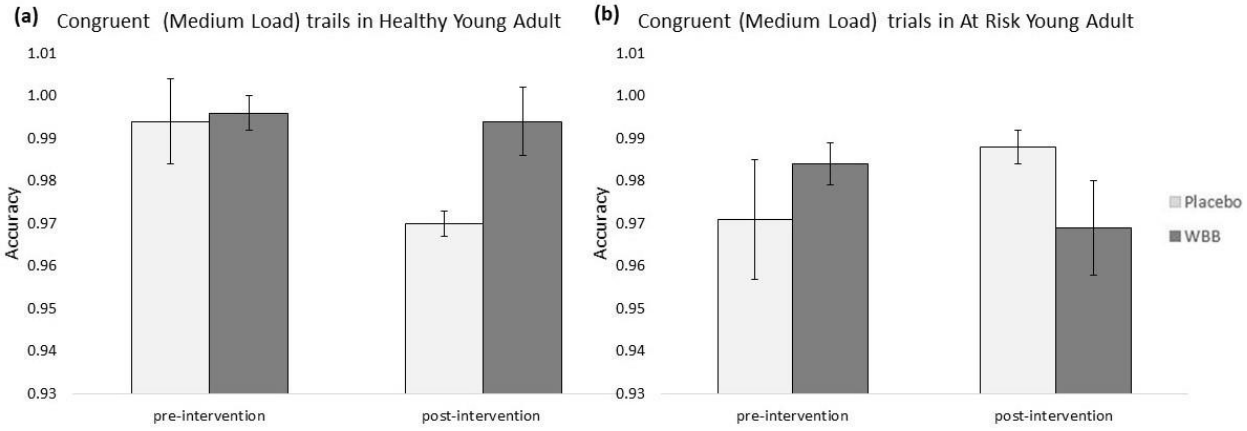

Figure 2. Mean (± standard error of the mean) Accuracy score on MANT in young adults aged 18-21 years: (a) Mean Accuracy score when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Accuracy score when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

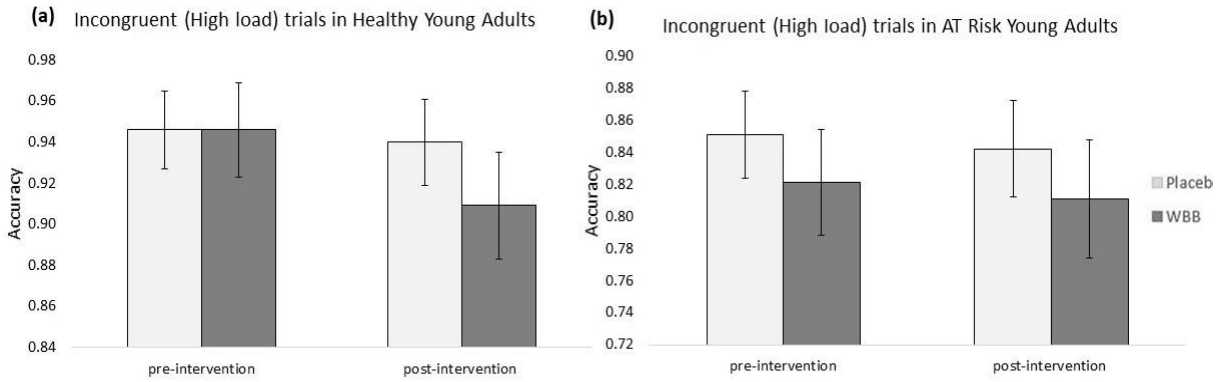

Figure 3. Mean (± standard error of the mean) Accuracy score on MANT in young adults aged 18-21 years: (a) Mean Accuracy score when incongruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Accuracy score when incongruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

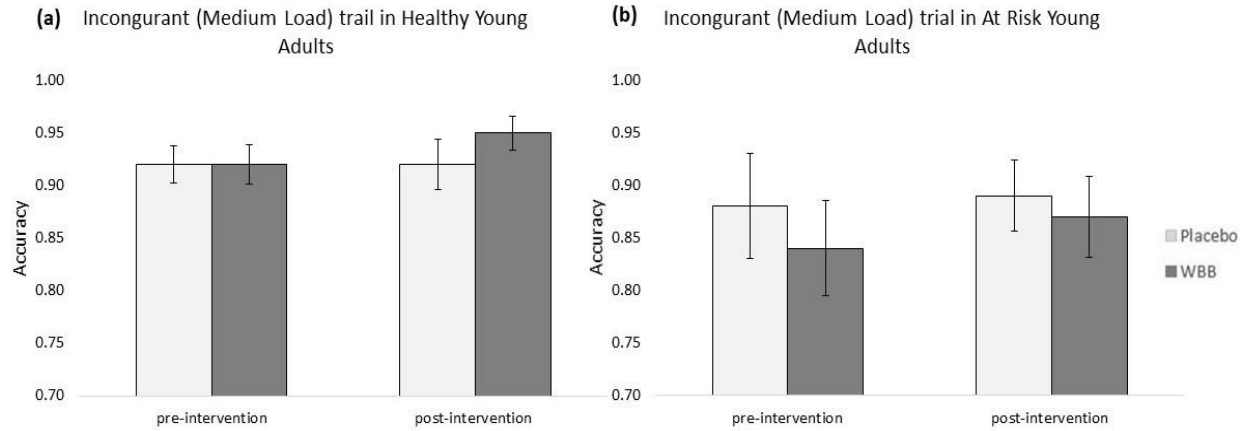

Figure 4. Mean (± standard error of the mean) Accuracy score on MANT in young adults aged 18-21 years: (a) Mean Accuracy score when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Accuracy score when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression**.**

12.8 Appendix 8: Study 4 Mean Reaction Time Assessed using Modified Attention Network Task (MANT)

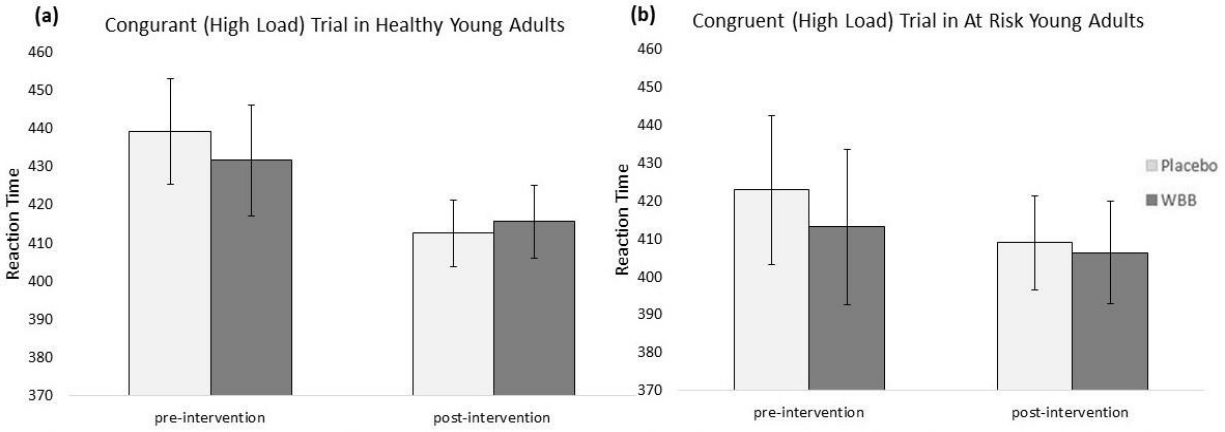

Figure 1. Mean (± standard error of the mean) Reaction Time on MANT in young adults aged 18-21 years: (a) Mean Reaction Time when congruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Reaction Time when congruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

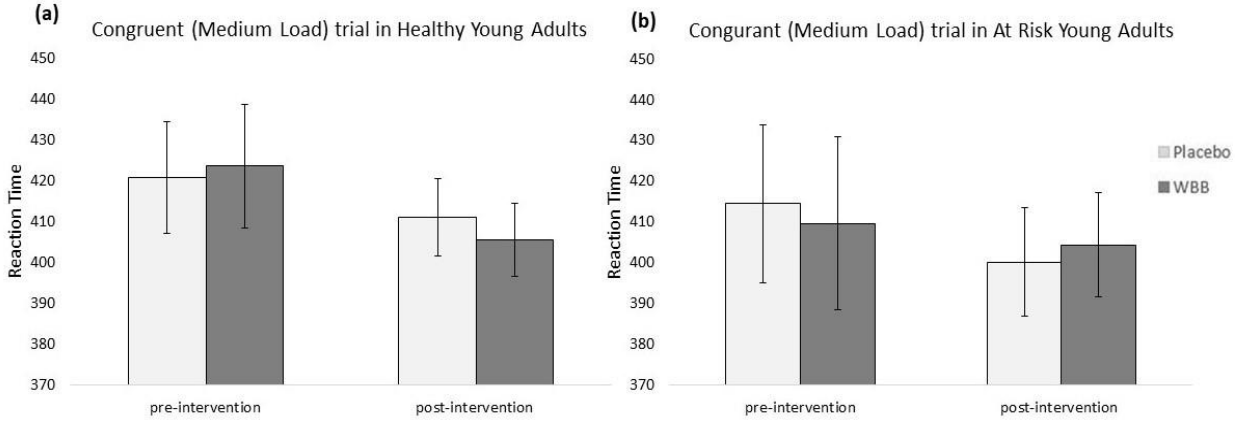

Figure 2. Mean (± standard error of the mean) Reaction Time on MANT in young adults aged 18-21 years: (a) Mean Reaction Time when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Reaction Time when congruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

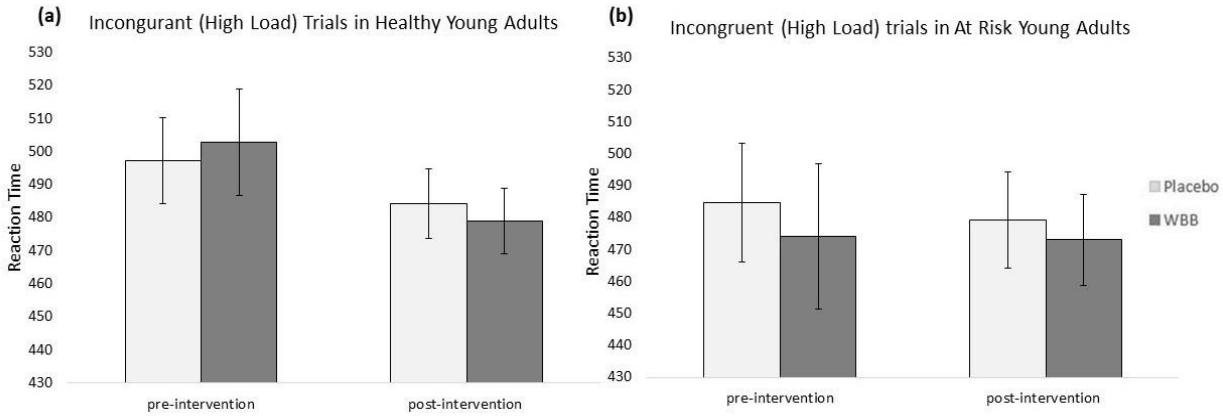

Figure 3. Mean (± standard error of the mean) Reaction Time on MANT in young adults aged 18-21 years: (a) Mean Reaction Time when incongruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Reaction Time when incongruent/high stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression

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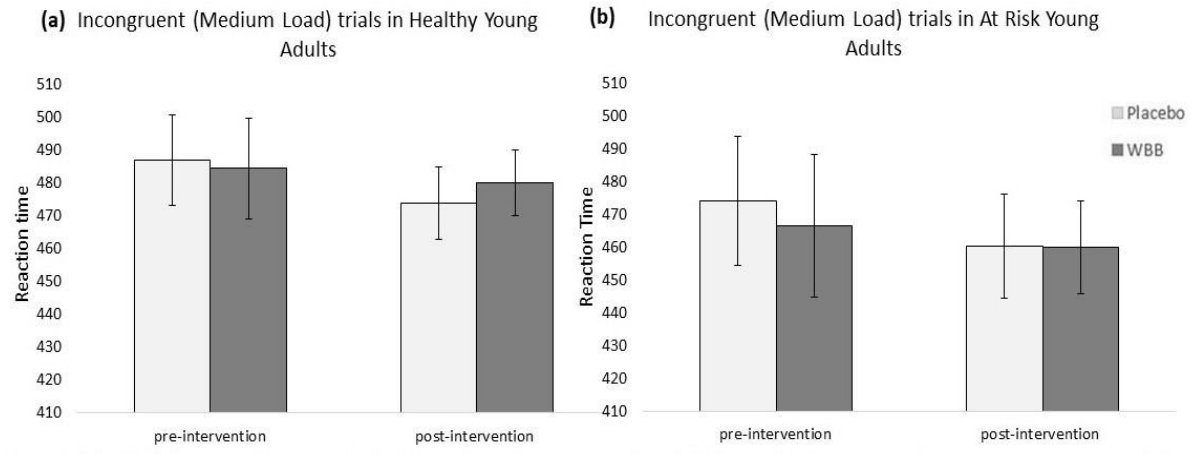

Figure 4. Mean (± standard error of the mean) Reaction Time on MANT in young adults aged 18-21 years: (a) Mean Reaction Time when incongruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Reaction Time when incongruent/medium stimuli were presented, pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

12.9 Appendix 9: Study 4 Mean Controlled Oral Word Association Task Scores

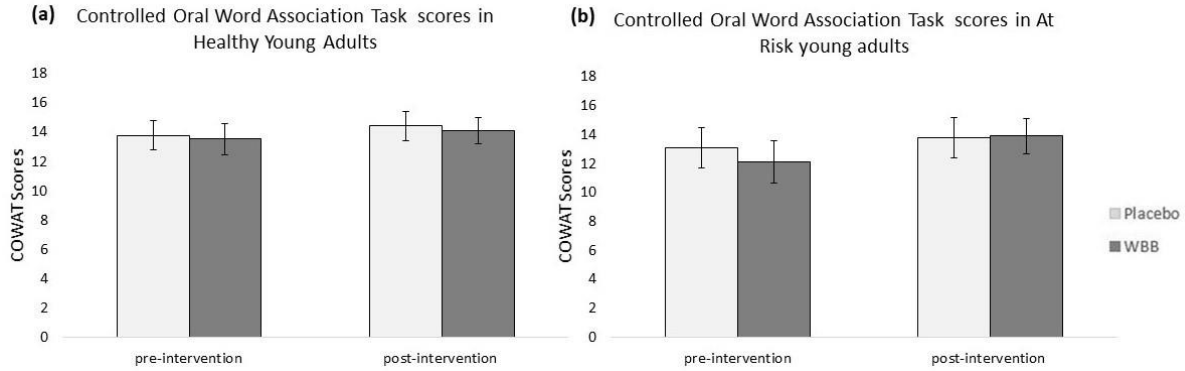

Figure 1. Mean (± standard error of the mean) Controlled Oral Word Association Task (COWAT) scores in young adults aged 18-21 years: (a) Mean COWAT scores pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean COWAT scores pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

12.10 Appendix 10: Study 4 Mean Keep Track Task Scores

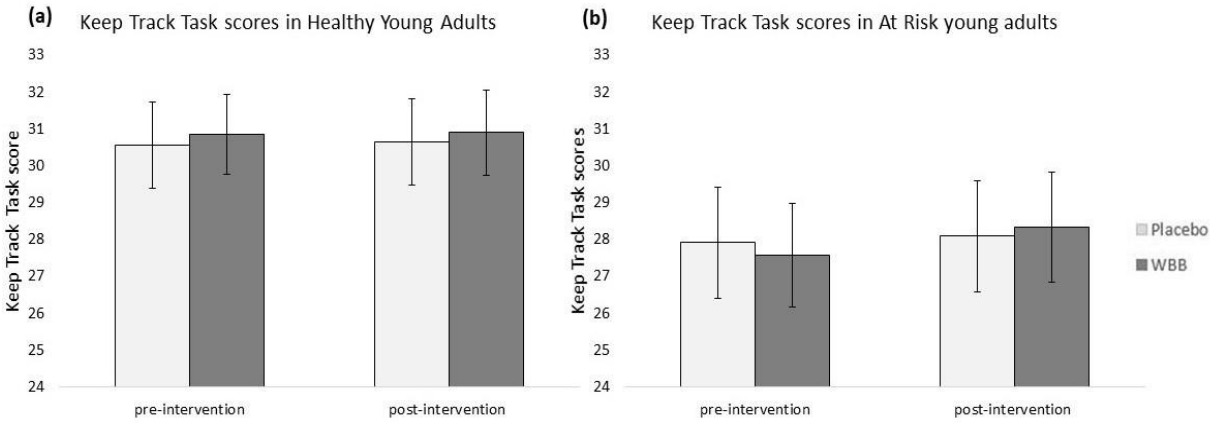

Figure 1. Mean (± standard error of the mean) Keep Track Task (KTT) scores in young adults aged 18-21 years: (a) Mean KTT scores pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean KTT scores pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression

12.11 Appendix 11: Study 4 Mean Serial 3 Scores

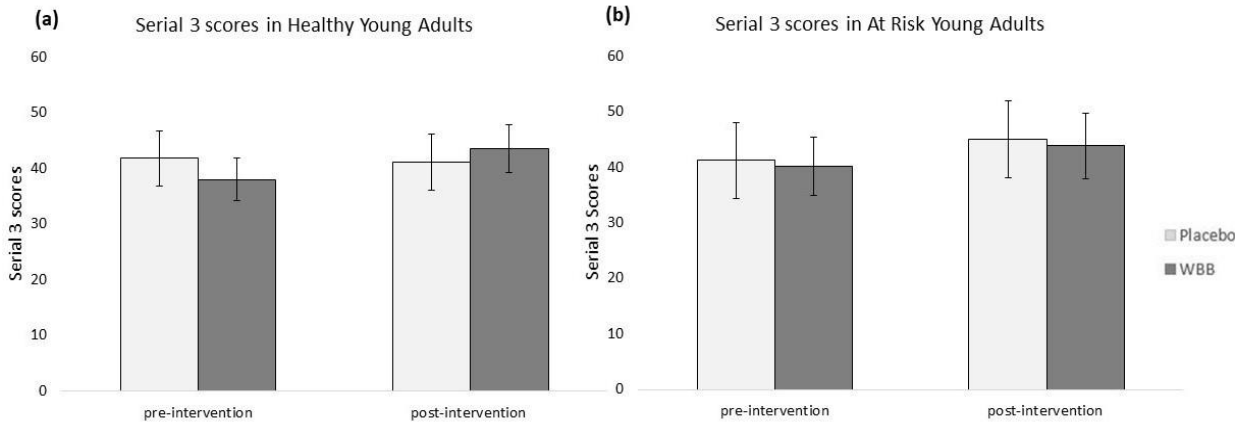

Figure 1. Mean (± standard error of the mean) Serial 3 scores in young adults aged 18-21 years: (a) Mean Serial 3 scores pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Serial 3 scores pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

12.12 Appendix 12: Study 4 Mean Serial 7 Scores

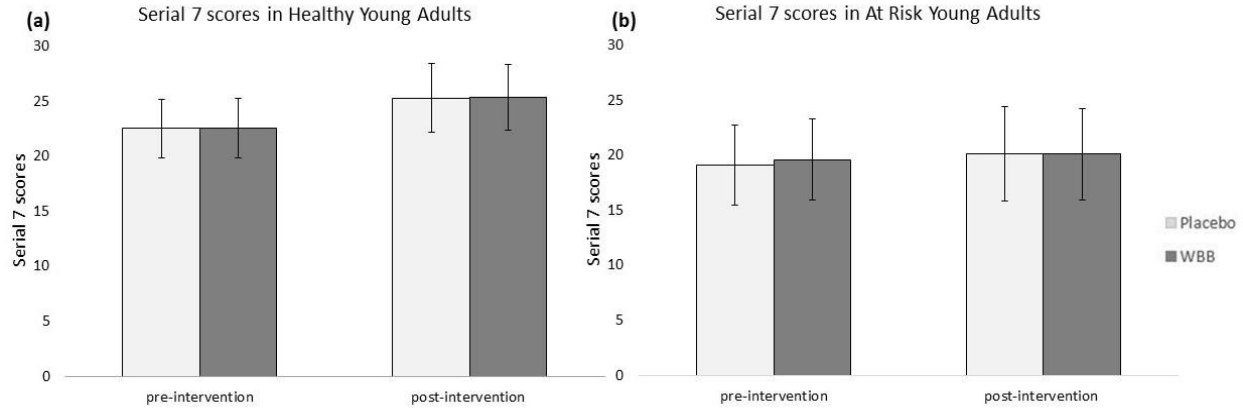

Figure 1. Mean (± standard error of the mean) Serial 7 scores in young adults aged 18-21 years: (a) Mean Serial 7 scores pre and post-consumption of placebo and intervention drinks in healthy young adults. (b) Mean Serial 7 scores pre and post-consumption of placebo and intervention drinks in adults with elevated symptoms of depression.

12.15 Appendix 15: Low Flavonoid Diet

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Polyphenol Free Diet

Please **avoid** drinking alcohol for **48** hours before **each** visit to the Nutritional Psychology Unit and for the duration of each study day.

Please **avoid** eating foods shown below for **24** hours before **each** visit to the Nutritional Psychology Unit and for the duration of each study day.

- All berries
- Fruit and vegetables (except potatoes)
- Fruit juices
- Jams and preserves
- Red wine
- Fruit teas
- Soy products
- Chocolate/cocoa
- Tea (black, green. earl grey etc)
- Coffee
- All high energy and/or caffeinated drinks, eg: Coca-Cola, Red Bull, Lucozade.
- All dietary supplements
- Alcohol (avoid for **48** hours)
- All Nuts

Foods you may eat include those shown below.

- Potatoes
- Rice
- Sweetcorn
- Mushrooms
- Carrots
- Bananas
- Pasta
- Meat/fish
- Dairy products

12.16 Appendix 16: University of Reading Ethics approval letters

12.16.1 University of Reading approval letter for Study 1

University of Reading

Coordinator for Quality Assurance in Research
Dr Mike Proven, BSc(Hons), PhD

Academic and Governance Services

Whiteknights House
Whiteknights, PO Box 217
Reading RG6 6AH

phone
fax
email

Professor Shirley Reynolds
School of Psychology and Clinical Language Sciences
University of Reading
RG6 6AL

26 May 2016

Dear Shirley

UREC 15/05: The relationship between nutrition and mood in adolescents. *Amendment favourable opinion*

Thank you for your application (emails dated 20 May 2016 from Sundus Khalid and including attachments refers) requesting and detailing amendments to the above project (*Inclusion of online recruitment and participation in Stage 1 of the project*). I can confirm that the UREC Chair has reviewed the request and is happy for the project to continue.

Yours sincerely

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

12.16.2 School University Ethics approval email for Study 2

From: Anastasia Christakou
Sent: 21 October 2016 10:52
To: PCLS Ethics
Cc: Sundus Khalid
Subject: Re: FW: New application 2016-114-SR

This application has SREC approval to proceed to UREC.
Signed forms attached.

Anastasia

12.16.3 School University Ethics approval email for Study 3

From: Anastasia Christakou
Sent: 08 May 2017 14:44
To: Sundus Khalid
Cc: PCLS Ethics; Shirley Reynolds; Claire Williams
Subject: Re: FW: Ethics extention 2016-110-SR

The amendment re study extension is approved by SREC.

Thanks,
Anastasia

12.16.4 University of Reading approval letter for Study 4

University of Reading

Coordinator for Quality Assurance in Research
Dr Mike Proven, BSc(Hons), PhD

Academic and Governance Services
Whiteknights House
Whiteknights, PO Box 217
Reading RG6 6AH

phone
fax
email

Professor Shirley Reynolds
School of Psychology and Clinical Language Sciences
University of Reading
RG6 6AL

28 November 2016

Dear Shirley

UREC 16/55: The Effects of Blueberry Anthocyanin Intervention on Cognitive functioning and Mood. *Favourable opinion*

Thank you for the application (email dated 15 September 2016 from Sundus Khalid and including attachments refers). On the basis of these documents I can confirm that the Chair is pleased to confirm a favourable ethical opinion.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here:

http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx

Yours sincerely

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Professor Claire Williams (Co-supervisor) Dr Laurie Butler (Head of School); Sundus Khalid (PhD student)

12.17 Appendix 17: Young People Information Sheets

12.17.1 Young People (under 16) Information sheets for Study 2

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Adolescents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (part 1)**

Hello,

We are inviting you to take part in a research project we are doing at your school.

Why is this project being done?

In this project we want investigate and understand how food and our diet relate to mood and feelings in young people aged 13 to 18 years.

Why have I been asked to take part?

You have been invited to take part because your school has agreed to help us with this project and because you are aged between 13-18 years, the age group we are interested in working with.

Do I have to take part?

Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

What will happen if I take part in this project?

We will come to your school and ask you to complete some questions about your mood and feelings. If you have trouble reading them, we will be able to help you. You would do the research in class, in a group.

Might anything about the project upset me?

The questions about your feelings may remind you of both happy and sad feelings. This is completely OK and normal. If you want to take a break or stop at any time, that is OK. We can talk about this at the time. You might also want to talk to your teachers or friends or parents about it.

Will my information be kept private if I take part?

Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do?

Before any research project is allowed to take place, it is checked by a group of people called an Ethics Committee. They make sure the project is ok to do. This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

What if I have more questions?

If you have any questions about this project, now or later, feel free to email or call us. You have the right to know everything and we are happy to tell you what you need to know.

Thanks,

Researcher: Sundus Khalid Email:

Phone:

Supervisors:

Prof Shirley Reynolds Email: Phone:

Dr Claire Williams Email: Phone:

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Adolescents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (Part 2 and 3)**

Hello,

We are inviting you to take part in a research project we are doing at your school.

Why is this project being done?

- To help us understand mood related problems in teens.
- To investigate the relationship between diet and mood in teens.

Why have I been asked to take part?

You have been invited to take part because your school has agreed to help us with this project and because your questionnaire scores in the 1st part means we would like you to participate in the 2nd and 3rd part of the study too.

Do I have to take part?

Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

What will happen if I take part in this project?

We will come to your school and ask you to complete some questions. These will include questions about what you eat, and how active you are. If you have trouble reading them we will be able to help you.

We will also invite you to participate in the 3rd part of the research which involves filling in an online food diary for 3 days. We will ask for your email address in order to contact you if interested.

What do I gain if I take part?

If you agree to participate and successfully complete the 2nd part, you will receive £10 and £20 upon completion of the 3rd part of the study (3 day online food diary).

Might anything about the project upset me?

The questions asked are not thought to be upsetting but we can talk about anything that might be upsetting. This is completely OK and normal. If you want to take a break or stop at any time, that is OK. You might also want to talk to your teachers or friends or parents about it.

Will my information be kept private if I take part?

Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do?

Before any research project is allowed to take place, it is checked by a group of people called an Ethics Committee. They make sure the project is ok to do. This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

Dr Claire Williams Email:

Phone:

12.17.2 Young People (over 16) Information sheet for Study 2

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Adolescents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (part 1)**

Why is this project being done? In this study we want to examine how mood and food are related, investigate and understand how food and our diet relate to mood and feelings in young people aged 13 to 18 years, which will help us better understand in understand the impact of nutrition on adolescent's mood

Why have I been asked to take part? You have been invited to take part because your school has agreed to help us with this project and because you are aged between 13-18 years, the age group we are interested in working with.

Do I have to take part? Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

What will happen if I take part in this project? We will come to your school and ask you to complete some questions regarding your mood and feelings. If you have trouble reading them, we will be able to help you. You would do the research in class, in a group.

Might anything about the project upset me? The questions about your feelings may remind you of both happy and sad feelings. This is completely OK and normal. If you want to take a break or stop at any time, that will be fine.

We can talk about this at the time if you wish or you might want to talk to your teachers or friends or parents about it.

Will my information be kept private if I take part? Would anyone else know I'm doing this? Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do? Before any research project is allowed to take place, it is checked by a group of people called an Ethics Committee. They make sure the project is ok to do. This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

What if I have more questions? If you have any questions about this project, now or later, feel free to email or call us. You have the right to know everything and we are happy to tell you what you need to know

Thanks,

Researcher: Sundus Khalid Email:

Phone:

Supervisors:

Prof Shirley Reynolds Email: Phone:

Dr Claire Williams Email: Phone:

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Adolescents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (Part 2 and 3)**

Why is this project being done? To help us better understand mood related difficulties in adolescents and to investigate how mood and food are related which will help us better understand in understand the impact of nutrition on adolescent's mood.

Why have I been asked to take part? You have been asked to take part because your school have agreed to help us with this project and your questionnaire scores in the 1st part of study means we would like you to take part in the 2nd and 3rd part of the study too.

Do I have to take part? Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

What will happen if I take part in this project? For the 2nd part of the study we will come to your school and ask you to complete some questions. These will include questions about your food consumption and how active you are. If you have trouble reading them, we will be able to help you.

We will also invite you to take part in the 3rd part of the research which involves filling in an detailed online food diary for 3 days. We will ask for your email address if you agree to take part in this.

What do I gain if I take part? If you agree to participate and successfully complete the 2nd part, you will receive £10 and £20 upon completion of the 3rd part of the study (3-day online food diary).

Might anything about the project upset me? The questions asked are not thought to be upsetting but we can talk about anything that might be upsetting. This is completely OK and normal. If you want to take a break or stop at any time, that is OK. You might also want to talk to your teachers or friends or parents about it.

Will my information be kept private if I take part? Would anyone else know I'm doing this? Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do? Before any research project is allowed to take place, it is checked by a group of people called an Ethics Committee. They make sure the project is ok to do. This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

What if I have more questions? If you have any questions about this project, now or later, feel free to email or call us. You have the right to know everything and we are happy to tell you what you need to know.

Thanks,

Researcher:

Sundus Khalid Email:

Phone:

Supervisors:

Prof Shirley Reynolds Email: Phone:

Dr Claire Williams Email: Phone:

12.17.3 Young People Information sheet for Study 3

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information Sheet

We would be grateful to you if you could assist us by participating in our study investigating the effects of flavonoids on cognitive functioning. Flavonoids are naturally occurring compounds that are widely distributed in plants and in plant-based foods and beverages (e.g. pulses, fruits like apple or blueberries, cocoa, etc). in the past decade there has been increasing interest in the potential health benefits associated with the consumption of flavonoid-containing foods. Research has suggested consumption of flavonoids can help improve cognitive functioning.

During the study you will attend 3 separate session. Each session will last 45 minutes. During each session you will be asked to first complete a set of questionnaires and complete a couple of computerised tasks. You will then be asked to consume a drink. In total, this section will take no more than 30 minutes. You will then be allowed to leave the laboratory and will be asked to return 2 hours later. Upon your return you will be asked to again complete the set of questionnaires and complete the computerised task. This will take no longer than 15 minutes. Your participation will take approximately 2.15 hours over the sessions in which after completing you will be awarded 2.25 credits through SONA.

Your data will be kept confidential and securely stored, with only an anonymous number identifying it. Information linking that number to your name will be stored securely and separately from the data you provide us. All information collected for the project will be destroyed after a period of 5 years from the completion of the project has elapsed. Taking part in this study is completely voluntary; you may withdraw at any time without having to give any reason. Please feel free to ask any questions that you may have about this study at any point.

Phone:

Supervisors:

Prof Shirley Reynolds Email:

Phone:

Dr Claire Williams Email:

Phone:

12.17.4 Young People Information sheet for Study 4

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Young People

The Effects of Blueberry Anthocyanin in Young Adults.

In this study we want to explore the effects of Blueberry Flavonoids on health and wellbeing in young adults.

What will happen if I take part in this project?

Screening:

Before participating in this study you will be asked to fill in a short health and mood questionnaires and return these to the experimenter via email. You may then be invited to participate in the test sessions.

Test sessions:

Test sessions will require you to be available to attend the Psychology department on **three** separate days and times that will be agreed with you in advance. Please let the Experimenter know if you cannot attend for any reason and the appointment can be rearranged.

For the 24 hours preceding these visits we ask that you follow a restricted diet (see attached), and do not partake in any vigorous physical activities. Please also refrain from eating or drinking for 2 hours immediately prior to each test session.

At the start of the first session, you will complete a food consumption questionnaire, before completing a short battery of computerised and interactive tasks. You will then be given a 250ml drink that may have a thick smoothie-like texture, after which you will be free to leave and return in 2 hours. We ask that you stay within the Department and do not eat or drink anything else other than water. After the break, you will complete the second set of tasks. Before leaving we

will also confirm your next visit date which will be one week later. The procedure will be exactly the same for all test visits, with the exception that you will be given a different drink.

Do I have to take part?

Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

Will my information be kept private if I take part? Would anyone else know I'm doing this?

Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries, you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do?

This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead.

What if I have more questions?

If you have any questions about this project, now or later, feel free to email or call us. You have the right to know everything and we are happy to tell you what you need to know.

Thanks,

Researcher: Sundus Khalid Email:

Phone:

Supervisors:

Prof Shirley Reynolds Email: Phone:

Prof Claire Williams Email: Phone:

12.17.5 Young People Information sheet for Study 5

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information for Young People

Project title: **The Effects of Blueberry Anthocyanin Intervention on Cognitive functioning and Mood.**

Hello,

We are inviting you to take part in a research project we are doing at your school.

Why is this project being done?

In this study, we want to explore the effects of Blueberry Flavonoids on health and wellbeing in adolescents. Flavonoids are naturally occurring compounds that are widely distributed in plants and plant-based foods and beverages (e.g. pulses, fruits such as apples or blueberries, cocoa etc.).

Why have I been asked to take part?

You have been invited to take part because your school has agreed to help us with this project and because you are aged between 13-16 years, the age group we are interested in working with.

Do I have to take part?

Whether or not you want to take part in this project is completely up to you. You do not have to do this. Also, it is completely OK if you chose to take part and then change your mind. You can withdraw from the project any time you like without having to give us a reason.

What will happen if I take part in this project?

We will come to your school and ask you to complete some questions about your mood and feelings. If you have trouble reading them, we will be able to help you. You would do the research in class, in a group.

A group of you will then be invited to the second part of the stud were your parents will prepare a drink for you every morning for 4 weeks. You will be asked to fill a short questionnaire to let us know your fruit and vegetable intake and complete a few computerised cognitive tasks and mood measures on the first day, two weeks and four weeks of intervention at PCLS laboratory in University of Reading or your school.

Might anything about the project upset me?

The questions about your feelings may remind you of both happy and sad feelings. This is completely OK and normal. If you want to take a break or stop at any time, that is OK. We can talk about this at the time. You might also want to talk to your teachers or friends or parents about it.

Will my information be kept private if I take part?

Everything you tell us as part of this project will be confidential and no one other than us will know what you have told us. The only exception is if you tell us anything that we think puts you or someone else at risk. If that happens, we would follow your school's procedures to keep you safe.

If you have any worries you can talk to us straight away. No paperwork will have your name on them. Everything you write will be kept in locked cabinets. Once we are done with our project, all the questionnaires will be shredded.

Did anyone else check if the project is OK to do?

Before any research project is allowed to take place, it is checked by a group of people called an Ethics Committee. They make sure the project is ok to do. This project is looked at by the University of Reading Research Ethics Committee and they are happy for us to go ahead. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

What if I have more questions?

If you have any questions about this project, now or later, feel free to email or call us. You have the right to know everything and we are happy to tell you what you need to know.

Thanks,

Researcher:

Jennifer Fisk Email:

Sundus Khalid Email: Phone:

Supervisors:

Prof Claire Williams Email: Phone:

Prof Shirley Reynolds Email: Phone:

12.18 Appendix 18: Parents Information Sheets

12.18.1 Parents' Information sheet for Study 2

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information about the Research for Parents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (Part 1)**

Purpose of the research: Depression and low mood are a common problem for young people, and it is important to find ways to reduce the risk of depression. There is some evidence that presence or absence of some nutrients found in our diets is associated with low mood and depression, but we know much less about the relationship between mood and diet in young people. In this study we want to examine how mood and food are related in young people, which will help us better understand in understand the impact of nutrition on adolescent's mood.

Why are we inviting your children to take part? Your child will be invited to take part in this study because their school has agreed to take part in this project and they are aged between 13-18 years, the age group we are interested to work with.

What will happen if my child takes part? Your child will be asked to complete some questionnaires regarding their mood and feeling in class.

Does my child have to take part? No. If you and your child want to take part, that's great. You don't have to do anything else. If your child does not want to take part, they can tell us when we visit the school, or they can ask you to let us know now. If your child is under 16 years and **you do not** want your child to take part in the study, please let us know.

This is an **OPT-OUT** study. Therefore, if you **DO NOT** want your child to participate in this study, please sign and return the attached forms or contact your child's school or us, either by email, phone, text, and conventional written materials. Our contact details are available at the

end of this letter. If you do not return this form or contact us in any way regarding this, it will be assumed that you are happy for your child to participate. If you want to Opt-out at any time that is fine. Also, your child is also free to opt-out at any time in the future.

What are the possible disadvantages and risks of taking part? We do not expect any disadvantages or risk to taking part in this study. Your child will answer questions about their feelings. It is possible that for some young people this might draw their attention to their feeling, and they might find this upsetting. We will work with the school to make sure that any children who are upset at the time are supported. In such a situation we would offer to stop the research immediately. The researcher who will be working with each class of children, under the supervision of the class teacher, is experienced at working with young people, has experience of working in an NHS clinic for children and young people, has full DBS clearance, and will receive regular supervision at the university.

The research will be carried out in school time with the whole class together during an appropriate period (e.g. PHSE or lunch break). Your son or daughter will not miss any lessons or spend time alone with the researcher.

What are the possible benefits? We hope that young people who take part in this research will enjoy the experience of being involved with science. After we do the research, we are happy to visit the school and talk about the research and the general effects of nutrition and exercise on health. In addition, taking part in this research will help us understand more about how food and diet affects the health and well-being of young people. This is important to help us improve their health and promote well-being. This school-based research will support the research we are doing in the NHS with young people who seek help for depression and anxiety We aim to use this information to improve clinical treatments.

What if there is a problem? If you have any concerns about any aspect of this study, you can ask to speak with Sundus Khalid, the researcher of this project. If you remain unhappy and wish to complain formally, you can contact one of the supervisors of this research, Prof Shirley Reynolds and Dr Claire Williams. Please see the last page for contact details.

Will taking part in the study be kept confidential? Yes. All information provided will be kept confidential. Questionnaires and other data will be identified by number only and kept in locked cabinets. Data entered onto computer will be anonymised and password protected. All paper records will be destroyed as soon as they are no longer needed with the exception of consent forms, which will be kept for 5 years before disposal.

Who has reviewed this study? To protect your interest, all research at University of Reading is reviewed by independent group of people called a Research Ethics Committee. Application for this research had been reviewed and approved by the University of Reading Research Ethics Committee. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

Contact details:

Researcher:

Sundus Khalid Email: Phone:

Project supervisors:

Prof Shirley Reynolds Email: Phone:

Dr Claire Williams Email: Phone:

If you have any questions please do not hesitate to contact us. We will be happy to tell you more about the research and to discuss any questions or concerns you may have.

Thank you for your time and cooperation.

Kind regards,

On Behalf of the Research Team at University of Reading.

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information about the Research for Parents

Project title: **The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (Part 2 and 3)**

Purpose of the research: To help us better understand mood related difficulties in adolescents and understand the impact of nutrition on adolescents' mood. There is some evidence that presence or absence of some nutrients found in our diets is associated with low mood and depression, but we know much less about the relationship between mood and diet in young people.

Why are we inviting your children to take part? Your child will be invited to take part in this study because their school has agreed to take part in this project and because their scores in Part 1 of the study makes them eligible to take part in the 2nd and 3rd part of this study.

What will happen if my child takes part? For the 2nd part of the study your child will be asked to complete some questionnaires, in class, regarding what they eat and how much exercise they take. Additionally, you the parent, will have to fill in the short demographic questionnaire attached.

We will also invite them to take part the 3rd part of the study. This would involve keeping a 3-day online food. This gives us much more detailed information about their diet but is more time consuming. If they are interested, they will be asked to provide contact details so that we can get in touch with them. We will ask them (and for under 16, a parent) to provide separate consent for the 3-day food diary study.

What will my child gain from participating? Your son/daughter will be receiving £10 for successfully completing part 2 of the study. If they are willing to keep a complete 3-day online food diary (part 3) they will receive £20 upon completion.

Your son/daughter will have an important opportunity to experience a real research study and if you or your child would be interested in finding out more about our results or other research, we would be more than happy to provide you with a summary.

Does my child have to take part? No. It's up to you and your son/daughter to decide whether to participate in this research or not. If you want to withdraw at any time you are free to do so. Also, your child is also free to withdraw from the study at any time in the future.

This is an opt-in study. So, if you **agree** that your child can take part, please sign and return the attach form. A separate consent form will be provided for the 3rd part of the study. We will not be able to include any child under 16 unless their parent has given written consent. Your child will also be asked if they are happy to participate. If not they are free to opt out themselves.

What are the possible disadvantages and risks of taking part? We do not expect any disadvantages or risk to taking part in this study. The researcher who will be working with each class of children, under the supervision of the class teacher, is experienced at working with young people, has experience of working in an NHS clinic for children and young people, has full DBS clearance, and will receive regular supervision at the university.

The 2nd part of the research will be carried out in school time during an appropriate period (e.g. PHSE or lunch break). Your son or daughter will not miss any lessons or spend time alone with the researcher.

The 3rd part of the study (3-day online food diary) can be completed in participants' own time anywhere with an internet access.

What are the possible benefits? We hope that young people who take part in this research will enjoy the experience of being involved with science. After we do the research, we are happy to visit the school and talk about the research and the general effects of nutrition and exercise on health. In addition, taking part in this research will help us understand more about how food and diet affects the health and well-being of young people. This is important to help us improve their health and promote well-being. This school-based research will support the research we are doing in the NHS with young people who seek help for depression and anxiety. We aim to use this information to improve clinical treatments.

What if there is a problem? If you have any concerns about any aspect of this study, you can ask to speak with Sundus Khalid, the researcher of this project. If you remain unhappy and wish to complain formally, you can contact one of the supervisors of this research, Prof Shirley Reynolds and Dr Claire Williams. Please see the last page for contact details.

Will taking part in the study be kept confidential? Yes. All information provided will be kept confidential. Questionnaires and other data will be identified by number only and kept in locked cabinets. Data entered onto computer will be anonymised and password protected. All paper records will be destroyed as soon as they are no longer needed with the exception of consent forms, which will be kept for 5 years before disposal.

Who has reviewed this study? To protect your interest, all research at University of Reading is reviewed by independent group of people called a Research Ethics Committee. Application for this research had been reviewed and approved by the University of Reading Research Ethics Committee. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

Contact details:

Researcher: Sundus Khalid Email:

Phone:

Project supervisors:

Prof Shirley Reynolds Email: Phone:

Dr Claire Williams Email: Phone:

If you have any questions, please do not hesitate to contact us. We will be happy to tell you more about the research and to discuss any questions or concerns you may have.

Thank you for your time and cooperation.

Kind regards,

On Behalf of the Research Team at University of Reading.

12.18.2 Parents' Information sheet for Study 5

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Information about the Research for Parents

Project title: **The Effects of Blueberry Anthocyanin Intervention on Cognitive functioning and Mood.**

Purpose of the research:

Flavonoids are naturally occurring compounds that are widely distributed in plants and plant-based foods and beverages (e.g. pulses, fruits such as apples or blueberries, cocoa etc.). Research has shown that short-term flavonoid intake improves cognition and wellbeing in both adults and children, however, no research has been carried out in adolescents. In this study, we want to explore the effects of Blueberry Flavonoids on health and wellbeing in adolescents.

Why are we inviting your children to take part?

Your child will be invited to take part in this study because their school has agreed to take part in this project, and they are aged between 13-16 years.

What will happen if my child takes part?

Part 1: Your child will be asked to complete some questionnaires about their mood and feelings in class. We will then invite around 120 boys and girls to the second part of this study.

Part 2: At this stage, your child will be randomly be assigned to either placebo or blueberry intervention. You (parent) will be given packs either blueberry powder or match placebo and instructions (written and video) on how to create the drink. You will be asked to prepare the drink for your child to consume every morning for four weeks.

Your child will be asked to fill a short questionnaire to let us know their fruit and vegetable intake and complete a few cognitive tasks and mood measures on the first day, two weeks and four weeks of intervention at PCLS laboratory in University of Reading or their school, discussed with you in advance.

Does my child have to take part?

No. If you and your child want to take part, that's great. For the first part of this study, you don't have to do anything else. If your child does not want to take part, they can tell us when we visit the school, or they can ask you to let us know now. If your child is under 16 years and **you do not** want your child to take part in the study, please let us know.

Part 1 is an **OPT-OUT** study. Therefore, if you **DO NOT** want your child to take part in this study, please sign and return the attached form or contact your child's school or us, either by email, phone, text, or in writing. Our contact details are at the end of this letter. If you do not return this form or contact us it will be assumed that you are happy for your child to participate.

The part 2 of this study you will be sent a separate **OPT-IN** consent form which we will require you to sign if you and your child would like to take part.

You or your child can choose to opt out of the study at any time. If you decide you do not want your child to be involved – just let us know and we will destroy any information they have given us.

What are the possible disadvantages and risks of taking part?

If they take part your child will answer questions about their mood and feelings. It is possible that for some young people this might draw their attention to their feeling, and they might find this upsetting. We will work with the school to make sure that if any child becomes upset that they are supported. In such a situation we would offer to stop the research immediately. The researcher who will be working with each class of children, under the supervision of the class teacher, is experienced at working with young people, has experience of working in an NHS clinic for children and young people, has full DBS clearance, and will receive regular supervision at the university.

The research will be carried out in school time with the whole class together during an appropriate period (e.g. PHSE or lunch break). Your son or daughter will not miss any lessons or spend time alone with the researcher.

What are the possible benefits?

We hope that young people who take part in this research will enjoy the experience of being involved in a science project. After we do the research, we are happy to visit the school and talk about the research and the general effects of nutrition and exercise on health. In addition, taking part in this research will help us understand more about how food and diet affect the health and well-being of young people. This is important to help us improve their health and promote well-being. This school-based research will support the research we are doing in the NHS with young people who seek help for depression and anxiety. This research improves our understanding of depression in young people and how to prevent and treat depression.

What if there is a problem?

If you have any concerns about any aspect of this study, you can ask to speak with any of the researcher, the researcher. If you remain unhappy, you can contact one of the supervisors of this research. You can also make a formal complaint if you are not satisfied by our response. Please see the last page for all of our contact details.

Will taking part in the study be kept confidential?

Yes. All information provided will be kept confidential. Questionnaires and other data will be identified by number only and kept in locked cabinets. Children's names or other identifying information will not be sorted by the researcher. Data entered into the computer will be anonymised and password protected. All paper records will be destroyed as soon as they are no longer needed with the exception of consent forms, which will be kept for 5 years before disposal.

Who has reviewed this study?

To protect your interest, all research at the University of Reading is reviewed by an independent group of people called a Research Ethics Committee. Application for this research had been reviewed and approved by the University of Reading Research Ethics Committee. Everyone working on this study has been through the formal Criminal Records Bureau Disclosure process and has been approved by the School of Psychology of the University of Reading to work with children and adolescents.

Contact details:

Researcher:

Jennifer Fisk Email:

Sundus Khalid Email: Phone:

Project supervisors:

Prof Claire Williams Email: Phone:

Prof Shirley Reynolds Email: Phone:

If you have any questions, please do not hesitate to contact us. We will be happy to tell you more about the research and to discuss any questions or concerns you may have.

Thank you for your time and cooperation.

Kind regards,

On Behalf of the Research Team at the University of Reading.

12.19 Appendix 19: Young People Consent and Assent

12.19.1 Consent Form

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Consent Form

Please circle all you agree with:

Have you read (or had read to you) the information about this project?	**YES/ NO**
Has somebody explained this project to you?	**YES/ NO**
Do you understand what this project is about?	**YES/ NO**
Have you asked all the questions you want?	**YES/ NO**
Have you had your questions answered in a way you understand	**YES/ NO**
Do you understand it's OK to stop taking part at any time?	**YES/ NO**
Are you happy to take part?	**YES/ NO**

If any answers are 'no' or you **don't** want to take part, **don't** sign your name!

If you **do** want to take part, please write your name and today's date:

Your name:

Date:

The person who explained this project to you needs to sign too:

Print name:

Sign:

Date:

12.19.2 Assent Form

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

ASSENT FORM FOR YOUNG PEOPLE

(To be completed by young people less than 16 years of age)

Please circle all you agree with:

Have you read (or had read to you) the information about this project?	**YES/ NO**
Has somebody explained this project to you?	**YES/ NO**
Do you understand what this project is about?	**YES/ NO**
Have you asked all the questions you want?	**YES/ NO**
Have you had your questions answered in a way you understand	**YES/ NO**
Do you understand it's OK to stop taking part at any time?	**YES/ NO**
Are you happy to take part?	**YES/ NO**

If any answers are 'no' or you **don't** want to take part, **don't** sign your name!

If you **do** want to take part, please write your name and today's date:

Your name:

Date:

The person who explained this project to you needs to sign too

Print name: Sign:

Date:

12.20 Appendix 20: Parental Consent

12.20.1 OPT-OUT Form

University of Reading
Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

OPT-OUT FORM

Researcher: Sundus Khalid

Supervisors: Prof. Claire Williams & Prof. Shirley Reynolds

*Please only complete and return this form if you **DO NOT** want your child to take part in this research.*

I **do not** agree with my child participating in this research.

Your child's name:
Class or form:
Your name:
Date:
Signature:

12.20.2 Parental Consent Form

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

PARENT CONSENT FORM

Title of Project: The Relationship between Nutrition and Mood in Adolescents: A Pilot Study (Part 2)

Researcher: Sundus Khalid

Supervisor: Prof. Shirley Reynolds & Dr Claire Williams

Dear Parent,

You have been sent this form as your child's scores in Part 1 of the study makes them eligible to take part in the 2nd part of this study. It involves your child completing questionnaires on their food consumption and physical activity. You (parent) are required to complete the questionnaire attached about ethnicity and socio-economic status. Students taking part in this part of the study will receive £10.

(Please initial each box)

I confirm that I have read and understand the Information Sheet for the above study and that I have had the opportunity to consider the information. ☐

I understand that my son/daughter's participation is voluntary and that we are free to withdraw at any time. ☐

I agree for my son/daughter to take part in the above study. ☐

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

Your child's name: _____

Your Name: _____ Date: _____

Signature: _____

Name of Researcher: _____ Date: _____ Signature: _____

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

PARENT CONSENT FORM

Title of Project: The Effects of Blueberry Anthocyanin Intervention on Cognitive functioning and Mood (Part 2)

Researcher: Jennifer Fisk, and Sundus Khalid

Supervisors: Prof. Claire Williams & Prof. Shirley Reynolds

Dear Parent,

We would like to invite your son or daughter to Part 2 of our research. Part 2 would involve you (parent) making drinks (blueberry or matched placebo) for your child to consume every morning for 4 weeks. completing questionnaires about their diet and physical activity. Your child will be asked to fill a short questionnaire to let us know their fruit and vegetable intake and complete a few cognitive tasks and mood measures on the first day, two weeks and four weeks of intervention at PCLS laboratory in University of Reading/ their school.

(Please initial each box)

I confirm that I have read and understand the Information Sheet for the above study and that I have had the opportunity to consider the information. ☐

I understand that my son/daughter's participation is voluntary and that we are free to withdraw at any time. ☐

I agree for my son/daughter to take part in the above study. ☐

I would **NOT** like my child to be contacted further for any related study ☐

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

Your child's name:

Your name:

Date:

Signature:

Name of Researcher: Signature:

12.21 Appendix 21: Debrief Sheets

12.21.1 Debrief sheet for study 4

University of Reading
Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Debriefing for a study: The Effects of Blueberry Anthocyanin on Cognitive functioning and mood in Young Adults.

This study was an investigation to examine how blueberry flavonoids, mood, and cognition are related, investigate and understand how flavonoids in our diet are related to mood and feelings, which will help us better understand the impact of nutrition on mood and mood disorders.

This was achieved by getting everyone completed the questions regarding their feelings of being sad or low and anxious in addition to general health questions. Participants were then selected based on the scores from these questionnaires and invited to the psychology department for the test sessions.

The first session was described as the first test session; however, this was just a practice session to get the participants familiar with the tasks and reduce practice effect.

The true aim of the study was hidden to encourage participants to answer truthfully and avoid the influence of social desirability or demand characteristics when asking them about their mood

We anticipate that participant when given the blueberry drink will perform better on the cognitive tasks and will have elevated positive mood and decreased negative mood.

If this is true, then our original suspicion that flavonoids can improve short-term mood in those with low mood and depression via improvements in cognition might be true.

Please feel free to contact me if you have any questions regarding this study.

THANK YOU AGAIN FOR YOUR CO-OPERATION.

Researcher: Sundus Khalid Email:

Phone:

Supervisors:

Prof Shirley Reynolds Email: Phone:

Prof Claire Williams Email: Phone:

12.21.1 Debrief sheet for study 5

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

Debriefing information: The Effects of Blueberry Anthocyanin Intervention on Cognitive functioning and Mood.

Thank you very much for taking part in this study. Our aim was to examine how flavonoids, which are found in many plants and fruits, especially blueberries, influence our thinking and mood. We want to understand more about any benefits of flavonoids in our diet on our mood and feelings and if this is related to improvements in our ability to solve problems. We hope that this study will help us better understand the impact of what we eat and how we think on our mood and well-being. This is because it is important to understand more about lifestyle factors that might promote well-being and prevent ill-health and diet is a very important part of this. Our hypothesis is that flavonoids have an effect on mood through their impact on executive functioning – something that is itself associated with low mood and depression. In the information sheet that we gave you before you and your parents took part in the study, we did not specifically describe our interest in mood at the start of the study. This was done to avoid the influence of social desirability or demand characteristics which may have influenced the results.

Again, thank you for your time and cooperation. If you have any questions regarding this study, please feel free to contact us.

Contact details:

Researcher:

Jennifer Fisk Email:

Sundus Khalid Email: Phone:

Project supervisors:

Prof Claire Williams Email: Phone:

Prof Shirley Reynolds Email: Phone:

12.22 Appendix 22: Support Information

12.22.1 Support information for 12-17-year olds

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

INFORMATION and SUPPORT

In this study, you completed some questionnaires relating to your mood and feelings and your diet. This was to help us with our research – thank you very much.

Sometimes filling in these questionnaires can make us aware of things we would like to discuss or get help with. We have given this leaflet to everyone who helped with the research. It suggests some potential sources of support if you feel it would be helpful to talk to someone. Often the first place to start is with a parent or another adult who is looking after you. They can often help you to get help from somewhere else.

- **Your General Practitioner**, who will be able to offer support or arrange for you to be referred to a counsellor.
- **Your Tutor** or **teacher you can trust,** they will be able to offer you guidance about other sources of support.
- **National organizations and helplines,** such as:
  - **The Samaritans.** You can call the Samaritans on 08457 90 90 90, Email: Jo@Samaritans.org. They provide confidential emotional support for those experiencing feelings of distress and despair. They are available 24 hours to listen is you are worried or feel upset and confused or just want to talk to someone.
  - **Childline**: 0800 11 11 Free 24hr confidential helplines for adolescents up to 19years of age. www.childline.org.uk

- **Young Minds**: Charity committed to improving the mental health of young people. www.youngminds.org.uk/
- **NHS Direct (England and Wales):** 111 Available 24hrs a day, all year round for health, advice, and reassurance.
- **MoodGYM:** https://moodgym.anu.edu.au/welcome Free interactive web program to prevent and reduce depression and low mood.
- **Papyrus:** 0800 068 41 41/ SMS: 0776 209 697 Email: pat@papyrus-uk.org. This is a UK national charity dedicated to the prevention of suicide and promotion of positive mental health and emotional wellbeing of adolescents.

- **Think Good-Feel Good**: Stallard, P. (2002). Chichester: John Wiley & Sons. Plus additional online resource: http://www.wileyeurope.com/go/thinkgoodfeelgood). Cognitive behaviour therapy workbook for children and adolescents.

- **Overcoming Teenage Low Mood and Depression: A five areas approach**. Dummett, N. & Williams, C. (2008). London: Hodder Arnold.

- **Am I Depressed And What Can I Do About it? A CBT self-help guides for teenagers experiencing low mood and depression**. Reynolds, S. & Parkinson, M. (2015). Oxford: Little, Brown Book Group Robinson.

12.22.2 Support information for 18-25-year olds

University of Reading

Department of Psychology and Clinical Language Sciences

University of Reading

Harry Pitt Building

Whiteknights Road

Reading RG6 6AL

INFORMATION and SUPPORT

In this study, you completed some questionnaires relating to your mood and feelings and your diet. This was to help us with our research – thank you very much.

Sometimes filling in these questionnaires can make us aware of things we would like to discuss or get help with. We have given this leaflet to everyone who helped with the research. It suggests some potential sources of support if you feel it would be helpful to talk to someone.

- **Your General Practitioner**, who will be able to offer support or arrange for you to be referred to a counsellor.

- **National organizations and helplines,** such as:
  - **The Samaritans.** You can call the Samaritans on 08457 90 90 90, Email: Jo@Samaritans.org. They provide confidential emotional support for those experiencing feelings of distress and despair. They are available 24 hours to listen is you are worried or feel upset and confused or just want to talk to someone.
  - **NHS Direct (England and Wales):** 111 Available 24hrs a day, all year round for health, advice, and reassurance.
  - **MoodGYM:** https://moodgym.anu.edu.au/welcome Free interactive web program to prevent and reduce depression and low mood.

- **SANE:** www.sane.org.uk charities concerned with improving the lives of everyone affected by mental illness. Helpline: 0845 767 8000, every day, 1:00pm-11:00pm
- **Anxiety UK:** works to relieve and support those living with anxiety disorders by providing information, support and understanding via an extensive range of services, including 1:1 therapy. Helpline 08444 775 774 Monday - Friday 9.30am - 5.30pm