DIETARY INTAKE, COGNITION, AND PSYCHOLOGICAL HEALTH

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Abstract

Dietary intake may be a modifiable lifestyle factor for improving psychological health. Research suggests that nutrition is associated with both mental health and psychological wellbeing. However, the independent contributions of specific dietary components including fruit and vegetable intake (frequency and quantity), or sweet and savoury snacking on a range of aspects of psychological health is yet to be established. Furthermore, cognition is a plausible psychological mechanism that may mediate this relationship. The acute effects of micronutrients in fruits on mood and affect have been measured, but the possible mood benefits of folate (a micronutrient found in vegetables) has received very little attention, and it is unclear how folate from vegetables compares to bioavailable supplementation. The aims of this thesis were to investigate the links between dietary intake and psychological health, to explore cognitive processes as a hypothetical mediator, and to examine the effects of folate on mood, affect, cognition, and psychological health. Results from the empirical studies showed that nutrient-rich fruit and vegetable consumption (separately) consistently predict symptoms of depression and positive psychological wellbeing (Chapters 3 and 4). How often fruit or vegetables are consumed may be more important than the overall quantity of intake (portions) to sustain these relationships over time. In addition, cognitive failures consistently mediated the cross-sectional relationship between nutrient-poor, savoury snacking and all aspects of psychological health, which highlights everyday memory errors as a novel mechanism. Further, overall diet quality may play an indirect role via cognitive failures for reducing stress. In Chapters 5 and 6, preliminary findings suggest that precise acute effects of bioavailable folate supplementation (L-methylfolate) may exist for the reduction of negative emotional states, including anxiety, negative affect, and negative mood. This thesis emphasises the potential of dietary intake as a target for enhancing psychological wellbeing, reducing cognitive failures and poor mental health.

Keywords: Fruit, Vegetables, Sweet snacking, Savoury snacking, Cognition, Mental Health, Psychological Wellbeing, Mood, Affect, Folate

Dedication

I dedicate this thesis to our beautiful son, Luca Atlas.

Your infectious giggle and sunny disposition have kept me inspired throughout the tough juggling of my responsibilities. You bring an entire new meaning to my life, thank you for spurring me on to see this through. Despite the sleep deprivation, your little face has always been a huge source of motivation to keep going.

My PhD journey has been much longer than I had intended it to be, but raising you is such a precious gift. So, here is the gift of “Mommy’s thesis is dedicated to you” – I hope all of this inspires you in the future.

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List of Abbreviations

BMI Body Mass Index

CFQ Cognitive Failures Questionnaire

DASS-21 Depression Anxiety and Stress Scale

F&V Fruit and Vegetables

FVI Fruit and Vegetable Intake

HADS Hospital Anxiety Scale

PANAS Positive and Negative Affect Schedule

PSS Perceived Stress Scale

RCT Randomised Controlled Trial

SD Standard Deviation

SF-FFQ Short-form Food Frequency Questionnaire

VAS Visual Analogue Scales

WEMWBS Warwick-Edinburgh Mental Wellbeing Scale

WHO World Health Organisation

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List of Publications from the research in this thesis

Peer-reviewed publications

Chapter 2: Tuck, N. J., Farrow, C., & Thomas, J. M. (2019). Assessing the effects of vegetable consumption on the psychological health of healthy adults: a systematic review of prospective research. *The American Journal of Clinical Nutrition, 110*(1), 196-211.

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Chapter 4: Tuck, N. J., Farrow, C. V., & Thomas, J. M. (2023). Frequency of fruit and vegetable consumption prospectively predict psychological health. *British Journal of Nutrition.* In prep.

Chapter 1 Introduction, Narrative Literature Review & Thesis Aims

1.1 Introduction

1.1.1 Public health significance of nutrition

One in four individuals report experience of a mental disorder during some period of their life (the World Health Organisation; WHO, 2020), thus a global Mental Health Action Plan for 2013-2030 was drawn up to bring attention to this concerning prevalence, this includes the targets of promoting mental health and preventing mental illness (WHO, 2021). By 2026, the National Institute for Health and Care Excellence (NICE) predicts that UK individuals requiring treatment for depression will increase by 17% to 1.45 million (NICE, 2023). Depression is the second cause of Disability Adjusted Life Years (DALYs) in the age category of 15-44 years (Reddy, 2010). Depression alone accounts for 4.3% of the global burden of disease and is among the largest single causes of disability worldwide, accounting for 11% of all years lived with disability, globally (WHO, 2013). In 2020, a rise in depression and anxiety disorders was observed alongside the COVID-19 pandemic; worldwide the number of cases of mental disorders rose dramatically, with an additional 53.2 million and 76.2 million cases of anxiety and major depressive disorders, respectively (Santomauro et al., 2021). There has been much discussion of the economic impact of poor mental health, with the cumulative global impact of mental disorders projected to reach US$16.3 trillion between 2011 and 2030, and this is higher than that of cancer, chronic respiratory diseases, and diabetes (Trautmann et al., 2016). Poor mental health is a significant threat to public health, associated with reduced wellbeing and quality of life (Prince et al., 2007), a shortened lifespan, lower educational attainment, and unemployment (Merlo & Vela, 2022). Psychological health is important in its own right, but it is also a major risk factor for chronic physical conditions (Bhattacharya et al., 2014). Thus, for policymakers, clinicians, and a significant proportion of the global population, addressing mental health is an important issue and a key public health challenge for the 21st century.

At the same time, poor dietary nutrition is rising. In the UK, the National Health Service (NHS) data shows that only 28% of adults consume the recommended 5-a-day, intake of sugar and saturated fat is higher than recommended levels, while intake of essential nutrients is insufficient (Statistics on Obesity, Physical Activity & Diet, England, 2020). Worldwide data shows that current dietary intake is high in energy, fats, and free sugars (those added to foods or drinks) and low in fruits and vegetables (Afshin et al., 2019). Moreover, less than 1% of the world's population score midway or above on the mean global Alternative Healthy Eating Index score, which ranges from 0 (least healthy) to 100 (most healthy) (Miller et al., 2022). This is a significant issue, as poor diet is a major contributor to morbidity (Ezzati & Riboli, 2012), all-cause mortality (Shivappa et al., 2017), premature mortality (Meier et al., 2019), and mental illness (Firth et al., 2020). Furthermore, poor diet is estimated to be responsible for 26% of global *preventable* mortality (Global Nutrition Report, 2021). Yet a synthesis of studies has shown that maintaining optimal intake levels of fruit and vegetables (2 portions of fruit and 3 portions of vegetables a day) can reduce the risk of total and cause-specific mortality (Wang et al., 2021).

Pharmacological and psychological treatment (e.g., cognitive behavioural therapy - CBT) are not always effective in resolving psychological health issues; both have achieved limited effectiveness in addressing the burden of poor mental health worldwide (Sarris et al., 2015). Meta-analysis evidence suggests that anti-depressant medication efficacy varies according to the severity of baseline depression, and the effect sizes are small (Cohen's d =0.11) for mild depression (Fournier et al., 2010). A conservative estimate states that 30% of patients with depression fail to respond to current medication therapies, and 45% discontinue use due to unpleasant side effects (Al-Harbi, 2012). A meta-analysis of psychotherapies for mild-to-moderate depression has identified a response rate of 48% across all forms of psychotherapy, compared to 19% in the control groups (Cuijpers et al., 2008). Psychotherapy can be effective, but is often expensive and inaccessible (Cuijpers et al., 2020). There is a need for the focus to shift from a treatment to a preventative model that implements protective measures across the lifespan, with the aim of targeting risk factors associated with mental illness (McDaid et al., 2019; Waechter et al., 2023). Longitudinal research suggests that individuals with a lower level of positive wellbeing are more likely to develop a mental illness (Wood & Joseph, 2010), and factors that promote positive wellbeing among individuals are critical in preventing mental illnesses (Burton et al., 2010; Kalra et al., 2012). Primary prevention includes health promotion (Singh et al., 2022); promotion and prevention are complementary and when combined, they can maximise positive outcomes (Saxena et al., 2002). One universal preventive strategy that targets the whole population, irrespective of individual risk, is maintaining a healthy lifestyle through exercise, sleep, and diet (Mrazek & Hagferty, 1994; Singh et al., 2022).

Dietary intake and nutrition have recently emerged as crucial targets for protecting mental health (Jacka, 2017; Marx et al., 2017). After an international consortium of mental health and nutrition experts, The Lancet published the recommendations that “Nutritional Psychiatry” should be incorporated into routine psychological health care (Sarris et al., 2015). This new field explores the evidence that nutrition through dietary intake, overall diet quality, and micronutrient supplementation, may be considered as one of several factors for promoting psychological health (Adan et al., 2019; Marx et al., 2017). The development of quality and consistent data is required to influence public health recommendations and clinical practice (Sarris, 2019). Identifying whether nutrition plays a direct or indirect role in mental health is complex, and it is difficult to prove that specific dietary components contribute to mental health through cause, benefit, or remedy due to the limited number of scientific studies to date (Adan et al., 2019). There is a need to conduct scientifically rigorous observational, intervention and experimental studies, that replicate and extend the current evidence base and explore diet as a determining factor of mental health, mood, and cognition (Jacka, 2017). Therefore, research that evaluates dietary intake as a new way to address the high prevalence, incidence, and health burden of mental illness (which is often comorbid with physical illness) is called for, and will inform preventative approaches (Collins et al., 2022). Daily lifestyle habits have been shown to impact both short-and long-term health and quality of life (Rippe, 2019), and dietary intake is a modifiable target for increasing nutrition, which may unlock a multitude of benefits to public health (Minich & Bland, 2013). Nutritional interventions may enhance psychological health across the lifespan, but research in this area is required to understand the impact of specific dietary components, and this will inform the development of dietary advice in relation to individual mental health.

1.1.2 Defining psychological health

The WHO defines psychological health as ‘a state of wellbeing in which the individual realises his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community’ (WHO 2001, p.1). An individual may experience poor psychological health without being diagnosed with a mental illness. This supports the Complete State Model of Mental Health (Keyes, 2003, 2005), that good psychological health is not merely the absence of illness, but rather a state of wellbeing (WHO, 2013). Mental illness is a health condition of emotion, thinking, or behaviour that causes distress and impaired functioning to be able to contribute to society (American Public Health Association, 2022). The promotion of positive psychological experience, such as psychological wellbeing is as important as protecting against symptoms of poor mental health, or mental illness (Linley et al., 2006).

Symptoms of depression, anxiety, stress, and positive mental wellbeing are all aspects of psychological health which can be considered along a continuum (e.g., Depression-Happiness as one continuum) ranging from negative to positive functioning (Joseph & Wood, 2010). Low levels of psychological wellbeing predict the incidence of depression (Wood & Joseph, 2010), and enhancing psychological wellbeing has been shown to reduce symptoms of depression (Seligman et al., 2006). Hence, promoting positive psychological wellbeing may act as a buffer against poor mental health, and increasing this in people at risk is effective prevention (Seligman, 2002). Therefore, in this thesis, the term psychological health is used to describe both negative (e.g., depression, anxiety, stress, sadness) and positive (e.g., positive psychological wellbeing, positive affect, happiness) psychological factors that contribute to an individual’s mental state. However, at times, this broader approach will be discussed as two facets: mental health and positive psychological wellbeing. Therefore, the following narrative review and studies will cover the links between dietary intake with both poor mental health and positive mental wellbeing, when referring to psychological health, informing the rationale for the empirical studies that follow.

1.2 Narrative Literature Review

1.2.1 Dietary intake assessment

The most used dietary assessment tool in previous observational and longitudinal studies is a food frequency questionnaire (FFQ), which assess the frequency of a food portion, and occasionally the quantity (total number of portions across the day), of consumption of various food groups (for instance, fruit, vegetables, whole grains, meat, fish, breads, snacks) (Jakes et al., 2004). Frequency of consumption is important because it implies habitual intake during the past month/s or year (Michels et al., 2005; Welch et al., 2005). FFQs capture how often a food item is consumed across the week or day, whereas quantity of intake measures average number of portions consumed in total per day, and frequency has been found to contribute more than portions to the variance in intake of most foods (Thompson & Subar, 2017). Thus, food frequency responses can be used to rank individuals according to their typical consumption of nutrients, foods, or groups of foods (Cade et al., 2002). Furthermore, FFQ data can be used to determine diet quality scores or adherence to dietary patterns (Molendijk et al., 2018). The Healthy Eating Index 2010 (HEI-2010; Guenther et al., 2013), is often used to assess diet quality based on adherence to dietary guidelines. Scores indicate the extent to which an individual reaches a dietary guideline on several important components, including various types of fruit and vegetables (F&V), wholegrains, and specific sources of fat or sugar.

Dietary patterns can be defined as “the quantity, variety, or combination of different foods and drinks in a diet, and the frequency with which they are habitually consumed” (Govindaraju et al., 2018, p. 2). When FFQs are used to determine healthy dietary patterns, this might include: a Mediterranean diet consisting of high intakes of fish, nut, fruits, vegetables (Hodge et al., 2013), Japanese diets (Nanri et al., 2010), or general ‘healthy’ diets, typically defined by a high intake of F&V, wholegrains, fish, and a low intake of processed food (Rahe et al., 2014). These more general healthy diets are reported using a variety of terms including: ‘health-conscious,’ ‘traditional,’ or ‘whole food diet.’ The components that make up these dietary patterns vary, and the number of factors retained in each pattern can be subjective. Interestingly, fewer observational studies identify the specific effects of fruit and vegetable intake (FVI) in isolation from the wider diet (Glabska et al., 2020), and if FVI is assessed in isolation, fruits and vegetables are usually combined into a single item (precluding any consideration of the potential differences between these two food groups). Furthermore, FVI is often assessed using self-reported habitual intake (as portions) extracted from the larger FFQs (Dharmayani et al., 2021). Frequency of F&V consumption is analysed less frequently, with the focus being on portions, serving size or quantity, and some studies simply assess if national intake guidelines are met or not. Less attention has been given to the frequency of consumption due to the combining of frequency and quantity measures into one estimate of average daily fruit and vegetable consumption, instead of analysing frequency (i.e., how many times in a day or week an individual consumes fruits and vegetables) and quantity effects separately (Ocean et al., 2019). This thesis focusses on and extends the literature of studies that explore fruit and vegetables as separate dietary components from the wider diet, not in combination, and will evaluate both the quantity (portions) and frequency of FVI separately throughout.

1.2.2 Diet quality or pattern and psychological health

Research reviews have identified a relationship between overall diet quality or healthy dietary patterns (e.g., the HEI-2010, Mediterranean diet) and depression (Lai et al., 2014; Psaltopoulou et al., 2013; Quirk et al., 2013; Sánchez-Villegas et al., 2009), whereby as diet quality or adherence increases, symptoms, or the incidence of depression decreases. High quality diets (typically including greater FVI) have been consistently associated with lower symptoms of depression (Klassen et al., 2009; Kuczmarski, et al 2010; O'Neil et al, 2014). Both adolescents and adults with better quality diets report fewer symptoms of depression and improvements in emotional functioning, both before and after adjustments for the influence of socioeconomic, family, and other potential confounding factors such as physical activity (Jacka et al., 2011, 2011; Jacka et al., 2013). Furthermore, in a sample of Australian women, a 'traditional' diet (including F&V consumption) was associated with reduced odds of developing unipolar or bipolar depression (Jacka et al., 2010). Additionally, a higher adherence to the Mediterranean diet (including frequent fruit and vegetable consumption) was significantly associated with reduced negative affect in a large population sample, with ages ranging from 21- to 101-years (Moreno-Agostino et al., 2018). Notably, previous research focussing on sub-clinical symptoms of depression have shown that a whole food dietary pattern is associated with lower depression scores, or symptoms (Akbaraly et al., 2009). Together this evidence suggests that diet (quality or patterns) could be a modifiable target for the prevention of poor psychological health. However, it is difficult to infer a causal effect of diet on psychological health from the correlational literature because (1) being depressed might lead to a lower intake of healthy foods, or (2) a lower intake of healthy foods may contribute to being depressed (or both).

Other aspects of psychological health have been examined in conjunction with diet quality or pattern, but depression has received the most attention (Lai et al., 2014; Quirk et al., 2013), with the least attention being given to positive aspects of psychological health (e.g., positive psychological wellbeing). Plant-based foods such as fruit and vegetables, are more often investigated in relation to positive psychological wellbeing (Rooney et al., 2013), and from the limited evidence, results have shown that whole dietary improvement, or a healthy dietary pattern (plant-based), is associated with positive mood in non-clinical adult samples (Ma et al., 2022; McMillan et al., 2011). By contrast, Moreno-Agostino and colleagues (2018) found no association between higher adherence to the Mediterranean diet and experiences of positive affect, operationalised as the average of positive emotions experienced, weighted by the duration (using the Day Reconstruction Method) (Kahneman et al., 2004). However, this study did find a relationship between higher adherence to the Mediterranean diet and increased evaluative wellbeing (the evaluation of one's life as having better subjective wellbeing), while accounting for depression and health status as confounding variables. Also, more specifically when the components of a Mediterranean diet were examined, a relationship between fruit consumption (portions per day) and positive affect was found. Thus, improving diet quality or adhering to a dietary pattern that includes regular and high intake of F&V may enhance psychological health, but more research that evaluates the specific benefits to positive psychological wellbeing is needed (e.g., positive mood, affect, subjective wellbeing) to determine the direction and causality of this relationship. Furthermore, analysis of dietary components may shed light on potential specific effects of some foods or habits over others.

Early cross-sectional research exploring the link between nutrition and mental health has been extended by an increase in longitudinal studies, and these support the notion that dietary intake influences psychological health, not vice versa (Berk & Jacka, 2019). However, few prospective cohort studies (controlling for relevant confounders) exploring diet and depression risk, or depression prevention trials, exist (Molendijk et al., 2018). Nonetheless, a systematic review of observational studies (cross-sectional, cohort, case-control) synthesised the research focussed on diet quality via healthy dietary indices and the risk of symptoms of depression (Lassale et al., 2019). Most of the studies included generally healthy adults and overall, there is a robust longitudinal link between both higher adherence to a Mediterranean diet and lower adherence to a pro-inflammatory diet and a reduced risk of depression. Despite fewer studies, the same association emerges for indices such as the HEI, and several other country-specific adherences to dietary guidelines scores. One of the studies included in the review included a large sample of non-clinical university graduates (n= 15,093), this showed that after 8.5 years, those adhering to a 'Mediterranean', 'healthy', or 'pro-vegetarian' diet had a reduced likelihood of developing clinical depression (diagnosis or use of an anti-depressant medication), compared to sub-optimal adherence (Sánchez-Villegas et al., 2015). Quirk and colleagues (2013) reviewed 25 studies on diet and depression which showed that dietary intake was assessed using validated or developed FFQs, 24-hour recall, diet history questionnaires, or at least eight different validated dietary scores or indexes. For the assessment of depression symptomology, several diagnostic and self-reported methods were used. Reviews highlight that the high level of heterogeneity in mental health assessment (i.e., some questionnaires only used in a single study, most focus on symptoms of depression, few use formal diagnosis) tends to limit clear conclusions as comparisons across studies is difficult (Rahe et al., 2014). Moreover, none of the evidence that reports the effects of dietary factors (i.e., dietary patterns, food groups, food and beverages, macronutrients, and micronutrients), on the prevention and treatment of depression among healthy or depressed adults, is rated as high-quality evidence (Xu et al., 2021). The aforementioned umbrella review of prospective meta-analyses found moderate quality evidence for diet quality or pattern scores (from eight meta-analyses), with low quality evidence for FVI from mostly cross-sectional data via a meta-analysis (Saghafian et al., 2018), thus a more detailed, higher quality evaluation of dietary factors in association with depression is called for.

The previous cross-sectional, prospective cohort and longitudinal research is insightful, but there is a need for experimental, randomised-controlled trials (RCT) to establish causality. Dietary interventions that aim to promote healthier dietary patterns have been associated with lower clinical depression, or symptomology using clinical assessment or subjective self-report (Francis et al., 2019; Opie et al., 2015, 2018). For instance, a Randomised Controlled Trial showed that healthy dietary changes (Mediterranean-style diet; Med Diet supplemented with fish oil) resulted in reduced symptoms of depression (Parletta et al., 2019). Additionally, the 'SMILES' (Supporting the Modification of lifestyle In Lowered Emotional States) 12-week trial identified that improving diet quality is effective for reducing symptoms of depression for those with a mood disorder (Jacka et al., 2017). Another 3-week RCT dietary intervention conducted in young adults with elevated levels of symptoms of depression, reported significantly lower depression scores in the dietary improvement group compared to the control group, after controlling for baseline scores (Francis et al., 2019). This reduced depression subscale score (from the Depression, Anxiety, Stress Scale, DASS-21) was maintained three months later. Firth and colleagues (2019) conducted a meta-analysis of dietary intervention RCTs on symptoms of depression and anxiety (n= 16, with 15 that investigated these effects on symptoms in non-clinical samples), and this included interventions with multi-components (e.g., exercise with diet). They concluded that healthy whole-diet transformation may improve depression scores (no effect was observed for anxiety), but interventions that focussed on individual foods or nutrients were not included. However, if positive changes to diet can induce positive changes in psychological health, it is important to identify which foods or food groups should be targeted to maximise effects, as there are significant barriers and difficulties to achieve whole-diet transformation, such as difficulty adjusting overall diet to favour healthy eating, avoiding unhealthy food at social activities, lack of time, and competing priorities (Seguin et al., 2014).

Establishing the magnitude of effects that dietary components have on psychological health will inform targeted, simple modifications to diet. Studies exploring the relationship between diet and psychological health commonly combine the role of FVI with other nutritious foods (Conner et al., 2017). Consequently, this limits the inferences about FVI specifically, and does not scrutinise or allow the unique contributions of F&V to be known. For instance, no association was found between Mediterranean diet scores and psychological health (CES-D scores), but when the components of this diet were analysed separately, 'plant food' intake (fruit, vegetables, and legumes) were negatively associated with symptoms of depression (Crichton et al., 2013), whereas no significant association was found for other Mediterranean dietary components (fish, cereals, dairy, eggs, and poultry). This highlights the importance of assessing the isolated impact of FVI on psychological health, which is not only simpler to modify than whole-dietary change, but could be driving the effects observed for diet quality. Therefore, the following two sections will discuss the specific relationship between FVI (separate to overall diet quality and pattern) and psychological health (mental health and positive psychological wellbeing).

1.2.3 Fruit and vegetable intake and mental health

Sufficient FVI is regarded as one of the key components of a nutritious diet (Trichopoulou et al., 2013). A growing collection of evidence suggests that the specific effect of F&V (separate to overall diet and other food groups) on improving mental health is promising (Wallace et al., 2020). For instance, a large population-based study (n= 296,121) observed across repeated cross-sectional samples, that greater F&V consumption was consistently associated with lower odds of psychological distress and depression diagnosis, after adjusting for a wide range of covariates (McMartin et al., 2013). Also, FVI was significantly associated with mental health status and a previous mood or anxiety disorder diagnosis across time points, whereby as FVI increased, the prevalence of mental health issues decreased. In support of this, a protective association between frequent consumption of fruit and vegetables and reduced odds of having a mood and/or anxiety disorder, independent of general health status, physical activity or alcohol use has been observed in a large study of Canadian immigrants (Emerson & Carbert, 2019). Furthermore, the Swiss Health Survey (n= 20,220) reported that participants fulfilling the recommended five portions-a-day of F&V had lower odds of being highly or moderately distressed, compared with those individuals consuming less F&V (Richard et al., 2015). Although not a direct measure of a particular mental health disorder, these psychological distress scores are indicative of the probability of psychiatric symptoms, as the measure has sensitivity for detecting DSM-IV Axis-I disorders in the general population. Contrastingly, a non-significant association was found between baseline FVI and psychological health status five years later in young adults (Takaoka & Kawakami, 2013). Other studies have also reported no association between mostly combined F&V consumption and mental health (Bhattacharyya et al., 2013; Kim et al., 2015; Wu et al., 2018). Inconsistencies could be due to critical methodological issues, including those related to how diet is measured (e.g., method or combining FVI), and shared variance between lifestyle behaviours, such as diet with exercise and smoking or alcohol use. Indeed, diet is only one component of a healthy lifestyle, and without measurement and control of confounding factors, it can be difficult to infer causality (i.e., that the link is not merely an artifact of a generally healthier lifestyle).

To date, research has shown that FVI consistently predicts psychological outcomes, but few studies have evaluated a range of aspects of mental health in non-clinical samples, with adjustment for other lifestyle behaviours. A systematic review of the association between habitual FVI and mental health in adults identified 61 studies in total (cross-sectional and prospective) (Glabska et al., 2020). Overall, the studies revealed that high total intake of F&V is associated with lower levels of psychological distress and symptoms of depression. This review combined observational data from different populations (healthy participants and those experiencing physical health issues across the lifespan), which revealed that studies mainly focussed on depression (n =33), and some studies assessed more than a single aspect of psychological health. However, stress (n =8), distress (n =8), mood (n =5), and anxiety (n =5), were investigated much less frequently. This synthesis of the literature recommended that future research should cover all aspects of mental health using similar methodology, to explore these relationships in more detail. Also, as this review combined different populations and designs, it is difficult to say if the impact of F&V intake is equal in healthy participants compared with those experiencing health complaints. Another recent systematic review focussed on the association between FVI and symptoms of depression in younger adults aged 15-45 years (Dharmayani et al., 2021). Twelve studies were identified, and five good quality studies adjusting for confounders found a negative relationship between FVI and symptoms of depression. Methodological differences between studies regarding the inclusion of confounders, for instance some have not reported any adjustments, or there are inconsistencies in the variables accounted for, which limits robust conclusions (Rooney et al., 2013). Thus, further research using a range of mental health measures and consistent inclusion of covariates is warranted, especially in generally healthy populations with the aim of preventing poor mental health.

Even though the relationship between FVI and stress and anxiety has been studied less, results are encouraging and consistent across populations. Previous research indicates that higher FVI is associated with lower levels of psychological stress (Roohafza et al., 2013; Saghafian et al., 2018). Li and colleagues, (2021) showed that frequency of F&V consumption and physical exercise predicted stress scores, after adjusting for gender differences in a large Chinese population (n = 8,147, mean age= 43 years). Moreover, a weak, negative correlation between habitual FVI and anxiety scores (DASS-21 subscale) has been reported in males (Beezhold et al., 2015). Likewise, a lower intake of F&V was associated with higher levels of anxiety in females, irrespective of age or social background (Cook & Benton, 1993). Although a bi-directional relationship may occur considering the complex interrelationship between dietary intake and mental health, for instance stress is a significant predictor of food selection, especially nutrient-poor food consumption, through emotional, comfort eating (Gibson, 2012). Experimental data shows that a stress-inducing manipulation (anticipated speech performance) increased the consumption of sweet, fatty (cake and biscuits) foods in emotional eaters (Oliver et al., 2000). In addition, students experiencing mild to moderate stress levels (measured using the DASS-21) are significantly less likely to consume F&V, compared with unstressed counterparts (Papier et al., 2015). Nonetheless, a prospective, negative relationship between FVI and anxiety disorder diagnosis has been observed (McMartin et al., 2013), whereby greater consumption across 12 months was associated with reduced odds of an anxiety disorder diagnosis. At the population level, there is an opportunity to intervene through interventions that increase FVI which may protect mental health, especially as high levels of stress and anxiety are known to precede clinical mental health disorder (Pine et al., 2001; Rice et al., 2004). There is significant co-morbidity between sub-clinical symptoms of anxiety and depressive or anxiety disorder (Kendler & Gardner, 1998). Hence, more attention should be given to the impact of FVI on these wider aspects of psychological health.

1.2.4 Fruit and vegetable intake and psychological wellbeing

As evidenced above, there has been much research on the negative outcomes of psychological health or poor mental health in relation to diet, including symptoms of depression, psychological distress, anxiety, and stress. In accordance with the WHO definition of psychological health, it is important that preventative strategies aim to reduce symptoms of ill-health, but also promote experiences of positive psychological wellbeing. Some studies have explored the link between FVI and psychological wellbeing, and increased positive mood or affect have been observed (Ford et al., 2013; Warner et al., 2017), in addition to higher optimism, self-efficacy, happiness (De Leon et al., 2022; Glabska et al., 2020), life satisfaction (Lengyel et al., 2009) and positive psychological wellbeing (Conner et al., 2017; Rooney et al., 2013). Approximately 36.9% of depressive cases first develop before 25 years of age (Solmi et al., 2022). Young adulthood (18–29 years) is a critical period for both psychological wellbeing and diet quality (Collins et al., 2022). Thus, investigating the relationship between dietary intake and positive mood in healthy, young adults is particularly important for protecting psychological health (Ma et al., 2022). Recently, a systematic review in young adults reported that the more often fruit and vegetables are eaten during a typical week (i.e., frequency of consumption), the better their psychological wellbeing is likely to be (Dharmayani et al., 2021). Furthermore, Winzer and colleagues (2018) using the GHQ-12 concluded that consuming fruit and berries on a daily basis is a determinant of stability in psychological health in the 18–29 years old age group. This highlights the importance of F&V as a food group for promoting psychological wellbeing. Assessing psychological wellbeing alongside mental health (instead of solely focussing on symptomology), may detect changes at the positive end of the scale, which might be sizeable at the population level.

Cross-sectionally, higher FVI predicts increased positive psychological wellbeing within a range of age groups. In the general adult population, across a series of cross-sectional research, Blanchflower and colleagues (2013) consistently reported that high F&V intake was associated with positive psychological health outcomes (including self-reported happiness, life satisfaction and psychological wellbeing). Specifically, across three data sets of randomly selected British participants (n= 80,000), a dose-response relationship was identified, whereby the portions of F&V consumed daily were associated with reduced mental health complaints (lower nervousness and feeling low) and increased happiness, life satisfaction, psychological wellbeing. These remained after the inclusion of confounding variables such as employment status, illness, disability, exercise, and social class. Moreover, the impact of FVI in predicting psychological wellbeing was unaffected by the inclusion of variables for the consumption of fish, meat, and alcohol. In support of these findings, a study with adolescents (n= 65,212) also found a positive linear association with positive psychological outcomes, which was the strongest for frequent FVI when compared with other healthy dietary behaviours, such as eating breakfast (Hong & Peltzer, 2017). Further, studies in young adults (aged 17-25 years old) observe a link between habitual FVI and higher levels of positive psychological wellbeing. For instance, eating F&V 'daily' or 'almost daily' was associated with higher scores of subjective happiness compared to those consuming F&V less frequently (Piqueras et al., 2011). Additionally, Lesani and colleagues (2016) reported a dose-dependent relationship between FVI (total portions per day) and happiness scores in university students. Similarly, a dose-dependent relationship between daily FVI and positive affect has been observed in young adults (Warner et al., 2016). In older aged cohorts (ages ranged from 82-97 years), increased FVI was associated with higher happiness scores measured by visual analogue scales (Jyväkorpi et al., 2018). Together, this evidence supports the positive impact of F&V consumption on psychological wellbeing, however, stronger inferences regarding causality can only be ascertained through prospective or experimental controlled studies.

Longitudinal research has shown that increases in FVI predict significant gains in happiness, optimism, and life satisfaction over the course of two years (Mujcic & Oswald, 2016). Furthermore, Ocean and colleagues (2019) provide prospective evidence across eight years that increasing both the frequency and quantity of FVI can enhance psychological wellbeing measured by the 12-item General Health Questionnaire (GHQ-12). Longitudinal mobile or web-based diary studies have found that FVI predict changes in daily positive affect (but not negative affect) over short-periods of time, such as 21 days (White et al., 2013). This suggests the possibility of very acute effects on positive mood states, that over time may protect psychological health. Results from another web-based diary study that involved the general population (but including hospital patients) support this, and have found that enhanced mood requires daily consumption of four or more portions of F&V (Chan et al., 2015). However, these studies do not report whether they control for baseline symptoms of poor psychological health. Another longitudinal study in UK adults over the age of 50 years, found that individuals experiencing higher levels of psychological wellbeing report regular FVI, and have an 11% reduced risk of intake falling below recommended levels at follow up (Boehm et al., 2018). Indeed, individuals that report higher wellbeing (e.g., positive emotions, optimism) may be more likely to report better general health (Diener & Chan, 2011), and frequent engagement in healthy lifestyle behaviours (Martín-María et al., 2018). Thus, the temporal sequence of habitual F&V consumption and increased psychological wellbeing, requires further investigation.

A recent systematic identified that few controlled intervention studies measuring the effects of FVI (separately or combined) on positive psychological wellbeing exist in healthy adults, in total, six studies (n =691) using an intervention design with a non-F&V control, and a validated outcome measure of psychological health were found (Appleton et al., 2023). Overall, studies reported small statistically significant increases in psychological wellbeing following F&V consumption. Due to the limited number of studies, the findings from whole-diet interventions that include increasing FVI, and measure the effects on a psychological health outcome, are also discussed here. During a 10-day whole-diet intervention (that increased FVI), results showed significant improvements in positive mood (e.g., vigour) (McMillan et al., 2011). Conversely, the 12-week SMILES trial (Jacka et al., 2017) found no improvements in wellbeing or mood after overall dietary improvement (results were only significant for depression), but this study recruited participants with a pre-existing mood disorder. Another 14-week whole-diet intervention with a non-clinical sample of post-menopausal women found no differences in positive mood scores (Torres & Nowson, 2012). However, these interventions did not exclusively manipulate FVI, which does not allow the impact of F&V to be quantified.

More specifically, Smith and Rogers, (2014) compared psychological wellbeing after delivering a 10-day snacking intervention (n =100). Participants were assigned to one of two snacking conditions: fruit snacks (an apple, large clementine, or banana) or crisps and chocolate snacks to eat each afternoon. During follow-up, subjective reports of emotional distress, fatigue, cognitive difficulties, emotional eating, and symptoms of depression and anxiety were assessed. The results showed that those in the fruit consumption condition reported significantly lower mental and physical health problems, compared with those in the chocolate and crisps condition. Those in the chocolate and crisp condition reported large increases in depression, emotional distress, and fatigue. Furthermore, a 6-week intervention involving 13 men with low vitamin C intake, showed that daily consumption of 2 kiwifruits resulted in improved mood (increased vigour and reduced total mood disturbance) compared to ½ a portion of kiwifruit (use of a comparator condition but no control; Carr et al., 2013). Moreover, Plaisted and colleagues (1999) provided participants with F&V for 8 weeks and observed increases in health-rated quality of life. Together, the results from the 10-day experimental manipulation, 6- and 8-week interventions suggest that healthy fruit snacking may influence psychological wellbeing outcomes, after a short period of time. However, evidence from interventions is limited so the differential effects of F&V is not quantifiable, and the sample sizes are small, with high risk of bias, thus stronger evidence is required to understand the impact and precise effects of F&V consumption on psychological health (Appleton et al., 2023).

1.2.5 The isolated impact of fruit and vegetables

There is a need for research to explore the individual contributions of F&V intake separately, as they may independently lead to different effects on psychological health. It has been shown that fresh fruit, salad/raw vegetables, and cooked vegetables are each independently associated with symptoms of depression in a large undergraduate sample (n= 3,706; El Ansari et al., 2014). A recent study analysed different types of vegetables and fruits in relation to symptoms of depression in 16,925 adults, and reported that specific types, and intake of both F&V (separately), are important for protecting psychological health (Sun et al., 2020). After adjustment for multiple potential confounders, and exclusion of participants with health conditions, the results suggested that intake of tomatoes and tomato mixtures, leafy green vegetables, berries, dried fruits, total vegetables, and total fruits were selectively inversely related to symptoms of depression. However, inconsistent findings have been reported. For instance, a study of 6,271 women (mean age= 55.45 years old) found that the odds of developing depression (incidence) over six years was significantly less in those who specifically ate more than 2 servings fruit per day, compared to those who consumed fewer (OR=0.82), and vegetable intake failed to prospectively predict the development of depression longitudinally (Mihrshahi et al., 2015). Moreover, Mujcic and colleagues (2014) found a weak, negative association between fruit intake and self-reported diagnosis of depression and anxiety, but no association was found for vegetable intake. By contrast, in older Taiwanese adults (> 65 years), only vegetable consumption was protective against depressive symptoms four years later, after controlling for baseline symptoms and cognitive status (Tsai et al., 2012). This is supported by findings from another study, that vegetable intake was associated with lower odds of a mood disorder diagnosis, however, fruit intake was not assessed (Meyer et al., 2013). Hence, future work needs to independently assess both FVI in relation to psychological health, to ensure meaningful comparisons can be made. If fruit or vegetables exert a preferential effect over the other for psychological health, it may represent a more effective dietary target.

Indeed, there is some evidence to suggest that vegetable consumption *may* have a superior influence compared to fruit. Studies highlight that specifically high consumption of vegetables alone have shown consistent benefits to physical health (e.g., reduced risk of cardiovascular disease, various cancers) (Appleton et al., 2016; Li et al., 2022). Hence, one study analysed which of the food components from the Mediterranean diet had the most potent benefits to mental health, and notably, vegetables (but not fruit) were identified as having a large impact (LaChance & Ramsey, 2015). Furthermore, when the nutritional composition of foods for the prevention and treatment of depression is evaluated, vegetables receive the highest Antidepressant Food Score based on the density of twelve anti-depressant nutrients, such as folate and iron (LaChance & Ramsey, 2018), vegetables are followed by organ meats and then fruits. However, some studies report non-significant associations between vegetable intake and depression, when comparing components of the Mediterranean dietary pattern (Sanchez-Villegas et al., 2009). Overall, a meta-analysis of eighteen studies exploring FVI and depression (cross-sectional and prospective data) found that for every daily 100grams increase in the consumption of either fruit or vegetables, an associated 3% reduction in the risk of depression is observed (Saghafian et al., 2018). However, this review identified two studies that reported non-significant associations for vegetable intake, whereas four studies reported non-significant associations for fruit intake. Nevertheless, a rigorous synthesis of prospective research to date, covering more aspects of psychological health (beyond depression) will bring new insights.

1.2.6 Snacking and psychological health

Previous research has considered overall dietary intake, but specific dietary behaviours should not be overlooked. For instance, snacking on nutrient-poor foods may be a habit that affects psychological health. Indeed, consumption of poor-quality foods is a factor known to increase the risk of depression (Kim & Lee, 2022). Furthermore, individuals may consume energy-dense foods to lift their mood, or as a replacement to preparing more effortful, balanced meals (Collins et al., 2022). Snacking between meals is a potentially problematic dietary habit for psychological health (Morshed et al., 2022). Snacking can refer to consumption of foods between main meals, and most of the time the foods consumed are poor in nutritional quality (e.g., chocolate bars, sweets, crisps, and savoury snacks) (Poobalan et al., 2014). A healthy diet is typically characterised by high intake of nutrient-rich foods such as F&V, and/or low intake of nutrient-poor foods such as processed, high-energy foods (e.g., cakes and biscuits), which are often consumed as snacks (Akbaraly et al., 2009; Waijers et al., 2007).

Frequent snacking on nutrient-poor, energy-dense, processed foods has been shown to have detrimental effects on psychological health. For instance, the higher the proportion of processed snack foods in the diet, the higher the risk of depression (Akbaraly et al., 2009). Frequent consumption of processed foods has been associated with an increased odds of depression five years later (CES-D odds ratio= 1.58). Additionally, higher intake of high-energy sweets (e.g., cakes and pastries) or savoury snacks (e.g., crisps) have been positively associated with symptoms of depression (Gómez-Donoso et al., 2020; Sánchez-Villegas et al., 2012), and stress levels (Mikolajczyk et al., 2009). Tolkien and colleagues (2019) completed a meta-analysis of 11 studies, containing a total of 101,950 participants at baseline, and the results showed that individuals consuming foods that represent a pro-inflammatory diet (e.g., sweets, processed or high fat foods) have a 1.4 increased likelihood of being diagnosed with depression or displaying symptoms, as opposed to those following an anti-inflammatory diet. Further meta-analysis evidence indicates that a 1-unit increase in the dietary inflammatory index is associated with a 6% increased risk of symptoms of depression (Chen et al., 2021). By contrast, four longitudinal studies identified that infrequent nutrient-poor food consumption (quantified as a low dietary intake inflammation index) is associated with lower incidence of depression (Lassale et al., 2019). Although, a large prospective study reported a non-significant relationship between overall diet quality and depression, after adjustments for covariates (Chocano-Bedoya et al., 2013). However, when dietary intake was analysed for 'inflammatory potential' and the inflammation index computed, this index predicted symptoms of depression over twelve years of follow up (Lucas et al., 2013). Together, the evidence suggests that a reduction in the consumption of nutrient-poor snack foods (alongside increasing FVI), may be crucial for protecting psychological health.

Previous research shows that the 'junk food' dietary pattern (energy-dense, low in nutritional value, high consumption of three food components: snack foods, processed foods, and sweets), positively predicts depression and anxiety scores, after adjusting for covariates in young adults (Rossa-Roccor et al., 2021). The variance explained was small (adjusted *R* squared for depression=.04 and anxiety=.01, respectively), but, the magnitude of the standardised regression coefficients was comparable to other covariates measured, such as social support which is known to be negatively associated with cognitive decline, depression, and anxiety (Anderson & Fowers, 2020). Recent results show that University students' junk food consumption is also significantly linked to increased stress, anxiety, and depression scores (ElBarazi & Tikamdas, 2023). This reinforces the potential deleterious effects of nutrient-poor dietary intake on psychological health.

Moreover, a high snacking dietary pattern (frequent snacking between meals) is associated with a higher likelihood of symptoms of depression over time, compared to a diet that scores low on this pattern (Le Port et al., 2012). The types of foods snacked on was not assessed, but snacking frequency is known to positively correlate with chocolate, crisp and biscuit consumption (Chaplin & Smith, 2011). The relationship between a snacking dietary pattern and symptoms of depression was stable over 10 years in both men and women (CES-D scores odds ratio=1.49-1.59). This longitudinal study demonstrates that reverse causality is a less likely explanation, whereby depression may promote snacking, due to the stable and long-term relationships observed (Le Port et al. 2012), although it is not possible to rule out that poor psychological health or low mood, leads or motivates individuals to consume snack foods more often. Emotional eating could be one factor explaining the association between symptoms of depression and consumption of sweet foods (Konttinen et al.,2010). Furthermore, it is not clear whether reductions in snacking could lead to enhanced psychological wellbeing, but by contrast, frequent snacking (8 or more times a day) has been shown to reduce psychological wellbeing (Aucott et al., 2014). Very few studies have explored the relationship between snacking and positive psychological wellbeing, some attention has been given to fast food and sugar-sweetened beverage consumption in children (Chang & Nayga, 2009). When considering eating contexts, Morshed and colleagues (2022) found that skipping meals is negatively associated with psychological wellbeing. Even though snacking was not captured by this study, skipping meals may be indicative of snacking behaviour. The intervention study by Smith and Rogers, (2014) found that daily consumption of chocolate and crisps in the afternoon resulted in increased symptoms of depression, emotional distress, and fatigue, but positive psychological wellbeing was not explored. More research is required to explore the nature of the relationship between snacking and psychological health, which is likely bi-directional. Greater consumption of "unhealthy" snacks, such as crisps and biscuits has been associated with negative feelings or worries about current and future health status (Chaplin & Smith, 2011), thus specifically evaluating the link between frequent nutrient-poor snack food consumption and psychological wellbeing is warranted.

1.2.7 Biological mechanisms

The micronutrients (e.g., complex carbohydrates, antioxidants, specific vitamins) found in fruit and vegetables (F&V) are known to positively influence a range of biological systems including: gut health, inflammation, and neurotransmission (Albenberg & Wu, 2014; Berk et al., 2013; Claesson et al., 2012; Rooney et al., 2013). Equally, the nutritional components of processed foods (e.g., saturated fats and sugars) have been shown to negatively influence both gut and brain systems (Lai et al., 2014; Molteni et al., 2002; Zainuddin & Thuret, 2012). For example, high fat diets in animal models have been shown to trigger inflammation (Kim et al., 2012) and impair neurocognitive behaviour independent of bodyweight (Francis & Stevenson, 2013; Noble et al., 2017). Also, higher intakes of F&V may displace and consequently reduce processed food intake, thus allowing for brain recovery (Rahe et al., 2014; Silva & Pogacnik, 2017). These biological systems have also been implicated in mental processes such as affect and mood regulation (Rosenblat et al., 2014), in addition to cognition (Kendig et al., 2021; Tooley, 2020; Wärnberg et al., 2009). The precise mechanism by which dietary intake effects psychological health is unknown, but research has started to explore how micronutrients may play a key role in improving memory (Bhoir et al., 2022) and balancing mood (Rucklidge et al., 2022). A systematic review of epidemiological studies show that folate and other B vitamins are specific nutrients that may protect psychological health (Sanhueza et al., 2013). Furthermore, a recent cross-sectional study identified that independent of confounding factors, total dietary score of methyl donor micronutrients (including folate) are negatively associated with psychological disorders (Lotfi et al., 2021). Thus, dietary intake is likely to impact psychological health via the processing of micronutrients in nutrient-rich or nutrient-poor foods.

It has been established that the specific bioactive micronutrients and processing by the body of F&V vary greatly, so individual differences in nutrient absorption and consequently the magnitude of effects may occur (Appleton et al., 2016, Liu, 2013, Slavin & Lloyd, 2012). Despite this, a range of hypothesised mechanisms by which FVI may impact psychological health have been suggested in the literature. For instance, F&V are high in flavonoids, which are potent antioxidants that may boost brain-derived neurotrophic factor (BDNF) levels (Neshatdoust, 2016). Positive experimental effects of flavonoid antioxidants (found in berries, apples, and green vegetables) have been observed on psychological health, including improvements in positive and negative mood (Amagase & Nance, 2008; Whyte et al., 2019). A blueberry drink (high in flavonoids) intervention study identified higher positive mood (no effect on negative mood) two hours post-consumption (Khalid et al., 2017). The effect was consistent across two populations involving children and young adults. Moreover, an epidemiological study showed that higher flavonoid intake was associated with lower risk of depression in older women (Chang et al., 2016). Additionally, reviews highlight the effects of flavonoid intake on cognitive performance, and these show that studies reported both acute and chronic cognitive benefits (Lamport et al., 2012; Bell et al., 2015; Macready et al., 2009).

Moreover, it is possible that antioxidants modulate mood and influence brain function through the reduction of oxidative stress, which has been associated with psychiatric disorders (Moylan et al., 2014; Ng et al., 2008). On the other hand, studies show that habitual consumption of nutrient-poor foods (high in saturated fat and sugar) are associated with increased inflammation in the body (Ljungberg et al., 2020), which in turn predicts the incidence of symptoms of depression and anxiety (Adjibade et al., 2019; Bergmans & Malecki, 2017; Wang et al., 2018). Notably, there is a growing awareness of the importance of the gut microbiota in psychological health. Dietary intake is considered among the most influential factors on the human gut microbiota from infancy to old age (Oriach et al., 2016). Research postulates that the protective health benefits associated with habitual FVI are due to positive changes in the gut microbiota profile, which is mediated by fibre that mechanistically reduces inflammation and oxidative stress (Dash et al., 2015; Fatahi et al., 2021; Glick-Bauer & Yeh, 2014). Fibre consumption from regularly eating F&V may be indirectly linked to fewer symptoms of depression (Saghafian et al., 2022). Therefore, the nutritional composition of foods (e.g., F&V and crisps or biscuits) may influence psychological health through a range of biological mechanisms related to reducing oxidative stress and inflammation.

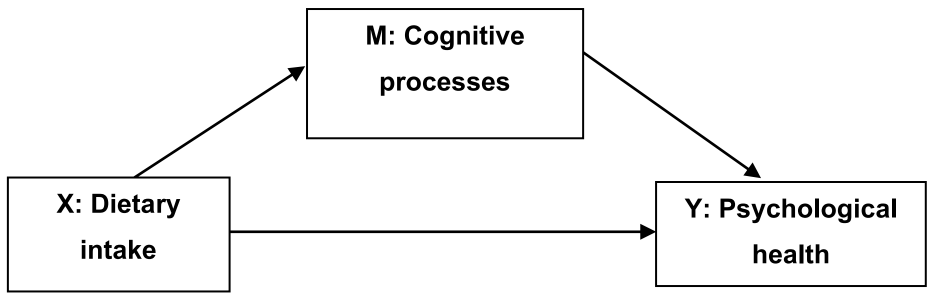
A specific micronutrient in vegetables, folate, has been observed to have antidepressant properties and its bioactive form (L-methylfolate) has neurochemical function (Martone, 2017). Folate (abundant in leafy green vegetables) may act on serotonin levels and monoamine metabolism (Bottiglieri, 2000; Bottiglieri, 2005; Woo et al., 2006). In fact, neurotransmitters (serotonin, dopamine, norepinephrine) are synthesised in the brain in a neurochemical cascade, which is partly regulated by L-methylfolate (Stahl, 2008). Low folate status has been found in studies of people with depression (Bender et al., 2017; Dimopoulos et al., 2007; Ng et al., 2009), and healthy adults with low folate intake have an increased risk of depression (Tolmunen et al., 2003). In support of this, a meta-analysis of observational studies reported that lower folate status was significantly associated with a higher prevalence of symptoms of depression (Gilbody et al., 2007a). Long-term low dietary folate is associated with increased negative mood variability in healthy males (Williams et al., 2008). Suboptimal intake of two micronutrients (folate and iron; the latter is also found in green leafy vegetables) were associated with an approximate 50% increased probability of mental health being perceived as poor, after the adjustment for numerous covariates (Davison et al., 2017). Furthermore, low folate levels are linked to a poor response to antidepressants, and treatment with folic acid is shown to improve response to antidepressants (Coppen & Bolander-Gouaille, 2005). Thus, folate supplementation has been suggested as an effective co-adjuvant in the pharmacological treatment of unipolar depression (Morris et al., 2008). A randomised, placebo-controlled trial showed that dietary supplementation of high doses of B-vitamins (involving B9 which is folate, combined with vitamin C and minerals) in healthy men led to improved ratings of stress, mental health, mood, and improved cognitive performance during challenging mental processing (Kennedy et al., 2010). Furthermore, five weeks of folate supplementation enhanced memory performance (recall and delayed recognition of words) when a battery of cognitive tests were completed by healthy adults (Bryan et al., 2002). Therefore, certain types of vegetables may provide a specific source of nutrients that protect psychological health, of which folate may play a pivotal role.

In addition to biological mechanisms, a psychological mechanism could be involved, known as “expectancy effects” (Kirsch, 1997; Sarris, 2019), whereby following a nutrient-rich diet may promote expected boosts to mood which in turn improves psychological health (Molendjijk et al., 2018). Both the positive psychological expectations and biological effects of consuming F&V may impact perceptions of psychological health (Sarris, 2019); hence it is difficult to delineate expectancies from biological effects. In nutritional research, psychological expectations have been observed in previous studies (Cassady et al., 2012; Lattimore et al., 2010), however more specific findings show that psychological expectancies did not mediate the relationship between FVI and psychological wellbeing (Conner et al., 2017). Whereas, Smith and colleagues (2022) found a weak partial mediation effect, whereby positive expectancies of the benefits of eating F&V, and actual habitual FVI, both independently and significantly accounted for variance in positive mood (but not negative mood). Thus, biological, and psychological mechanisms may explain the links between dietary intake and psychological in general adult populations, but firm conclusions cannot be made due to the present lack of data (Glabska et al., 2020). Further exploratory work to clarify these plausible mechanisms will enable greater understanding of what mediates cause and effect.

1.2.8 Cognitive processes as a mediator

The psychological mechanisms by which diet affects psychological health have received very little attention, even though a review highlights the potential role of cognitive processes as a mediator (Rao et al., 2008). Further investigation that explores how diet affects psychological health via cognitive factors will bring new insights. The following subsections will cover firstly the link between dietary intake and cognitive processes, secondly the relationship between cognitive processes and psychological health, then how these may combine, and finally the specific cognitive processes that may play a mediating role. As per Figure 1.1, cognitive processes will be explored as a hypothetical mediator in the relationship between dietary intake and psychological health.

Figure 1.1 Cognitive processes as a hypothetical mediator in the relationship between dietary intake and psychological health.



1.2.9 Dietary intake and cognitive processes

Both nutrient-poor and nutrient-rich dietary intake has been shown to impact cognitive processes. Factor analysis of dietary intake from a large UK FFQ dataset, established that 'whole food' (including F&V, and fibre intake) and 'processed food' (e.g., chocolates and crisps) consumption predicted a range of cognitive processes after adjusting for covariates (Akbaraly et al., 2009). In a review of studies, Lamport and colleagues (2014) observed statistically significant benefits of FVI on cognitive performance in 80% (20/25) of studies, including better global cognitive function, or prevention of cognitive decline such as reduced memory function. All but one study sampled older adults aged over 45 years, and none explored the acute effects of vegetables (all involved fruit juice consumption) over short periods of time of less than four months. Moreover, Polidiri and colleagues (2012) found that healthy older adults (mean age=70.8, SD +/-14.9 years) consuming the recommended five portions of F&V a day demonstrated higher levels of serum antioxidant micronutrients, lower levels of oxidative stress biomarkers and better cognitive performance, in comparison with healthy older adults consuming one daily portion or less of F&V. Conversely, habitual consumption of nutrient-poor foods (e.g., a Western-style diet, sugar, or saturated fats) is associated with poorer cognition, across age groups and after controlling for covariates (Stevenson, 2016). It is not clear whether benefits are observed throughout the lifespan, in healthy adults, or how immediate the benefits or detrimental effects on cognitive function occur.

Longitudinal studies identify that habitual nutrient-rich dietary intake is associated with better cognitive performance, whereby as diet quality increases (measured across 46 food groups e.g., fruit, vegetables, legumes) so does verbal memory and executive function, after adjusting for energy intake and demographic factors (Zhu et al., 2015). F&V consumption may have a protective effect on memory and executive function (Sabia et al. 2009), and evidence shows that people with high intakes perform better on global cognitive tests and are less likely to exhibit cognitive decline (Gillette-Guyonnet et al., 2007). A dose-response meta-analysis showed that an increment of a 100-

g per day increase in F&V consumption was related to a 13% decrease in cognitive impairment and dementia risk (Jiang et al. 2017). Moreover, Sabia and colleagues (2009) reported that consuming less than two portions of F&V per day at baseline, was associated with significantly worse memory and executive function at 11-year follow-up, and this association strengthened over time. Furthermore, Kang et al. (2005) analysed baseline food frequency intake and found that specifically higher cruciferous (leafy green) vegetable intake was associated with better performance on a battery of cognitive tests at follow-up 20 years later (dietary assessment was conducted every four years.) On the other hand, a prospective association between saturated fat intake and cognitive decline is observed, especially in memory (Eskelinen et al., 2008; Morris et al., 2004, 2006). Thus, memory performance appears to be consistently linked to dietary intake.

Poor dietary intake may play a role in accelerating adverse physiological changes relevant to cognitive processes as we age. Jacka and colleagues (2015) identified that both lower scores on a nutrient-dense dietary pattern and higher scores on a nutrient-poor dietary pattern, were independently associated with smaller left hippocampal volumes (involved in memory function) in older adults. One daily portion of leafy green vegetables has been shown to slow cognitive decline associated with aging (Morris et al., 2018). A systematic review concluded that when FVI is analysed separately in older adults (6 studies), increased intake of vegetables is associated with a slower rate of cognitive decline, but the association with fruit is uncertain (Loef & Walach, 2012). However, in middle-aged adults, no relationship emerged between habitual diet (Mediterranean diet adherence known to be high in FVI) and cognitive function (Crichton et al., 2013). Therefore, effects may be more readily observed in older populations due to the margins for prevention of age-related cognitive decline (Adan et al., 2019; Prenderville et al., 2015), but research in younger adult samples is warranted

Laboratory experiments in young adult samples investigating the effects of high fat/high sugar intake on cognition (Reichelt & Rank, 2017; Attuquayefio et al., 2017; Stevenson et al., 2020), combined with manipulations in animal models (Beilharz et al., 2014-2016; Kanoski et al., 2007), support the conclusion that frequent consumption is associated with cognitive impairment. Diet-induced memory impairments have been linked to anxiety-like behaviours in animal models (Peris-Sampedro et al., 2019). Furthermore, higher consumption of nutrient-poor foods and lesser consumption of nutrient-rich foods, has been associated with poor executive functioning (Allom & Mullan, 2014; Hall et al., 2014). In young adults, processed food intake, saturated fat and sugar consumption are associated with poorer hippocampal-dependent learning and memory performance (Brannigan et al., 2015; Francis & Stevenson, 2011; Gibson et al., 2013). Self-reported intake of processed food, saturated fats or sugars show that higher intake is associated with poorer memory (e.g., recall errors, memory retention) (Taylor et al., 2021). Collectively, research suggests that nutrient-poor dietary intake may induce detectable cognitive impairment in young adults; memory and executive functioning deficits have been observed, which is consistent with the evidence base in older adult samples.

More specifically, sweet, and savoury snacks are one of the main processed foods high in sugar or saturated fats, but the relationship between snacking habits and cognitive performance is mixed. In university students, sweet or savoury snacking throughout the day were not associated with daily functioning, namely affect and academic performance (Flueckiger et al., 2017). On the other hand, snacking has been shown to enhance cognitive performance (Miller et al., 2013), or no association between snacking habits and cognitive performance has been observed (Smith, 2011). Snacking may have positive effects on cognitive performance; several small meals at frequent intervals (“grazing”) is associated with better verbal reasoning accuracy than eating fewer and larger meals at longer intervals (Hewlett et al., 2009). However, the previous literature typically does not evaluate the types of snacks consumed (nutrient-poor or nutrient-rich), and the limited research exploring snacking has been experimental, rather than assessing intake in a real-world, naturalistic context over time. Studies that explore the frequency of sweet and savoury snacking and psychological health in young and general adult populations, will add to the current, lacking evidence base (Francis et al., 2019). This should include a range of covariates, such as age and BMI which are also known to correlate with cognitive impairment (Smith et al., 2011), to establish whether a robust relationship exists between dietary intake and cognition.

1.2.10 Cognitive processes and psychological health

Impaired cognitive processes are known to reduce psychological health by increasing symptoms of poor mental health, and reducing psychological wellbeing (Austin et al., 2001; Knight et al., 2020). Research suggests that psychological health is contingent upon cognitive processes; even subtle cognitive deficits are significantly associated with psychological wellbeing, after controlling for depression (Gates et al., 2014). A review of cognitive impairments in young adults with major depression and anxiety disorders showed that cognitive dysfunction is common, which emphasises the interplay between cognition and psychological health independent of age (Castaneda et al., 2008). Furthermore, evidence suggests that impaired executive functioning has been associated with the maintenance of depressive symptoms, including attentional deficits and impaired inhibitory control, which may be associated with clinical levels of perseveration and rumination (Gohier et al., 2009; Marazziti et al., 2010). In addition, research shows that attentional bias to negative stimuli contributes to high levels of anxiety and has been implicated in depression susceptibility (Clasen at al., 2013; Lichtenstein-Vidne et al., 2017). Indeed, recent evidence showed that attentional bias was significantly correlated with mood fluctuations in daily life; a negative bias was associated with increased temporal instability of anxious mood (Iijima et al., 2017). Moreover, Beevers and Carver (2003) showed that negative attentional bias following a negative mood induction was associated with increases in dysphoria seven weeks later, representing susceptibility to depression. Cognitive deficits specifically in immediate or delayed memory, have been associated with lower mental (but not physical) quality of life in acutely depressed and long-term depressed individuals, compared to healthy controls (Knight et al., 2020). Overall, evidence shows that a range of compromised cognitive processes predict poorer psychological health. There are multiple potential cognitive mediators between the relationship of dietary intake and psychological health, however, cognitive deficits related to memory and inhibitory control are consistently linked to dietary intake and psychological health (Eskelinen et al., 2008; Lamport et al., 2014; Sabia et al. 2009), thus may represent critical targets. Considering the findings demonstrating a relationship between dietary intake and cognition, together with evidence that cognitive impairments impact psychological health, it is plausible that the influence of dietary consumption on psychological wellbeing and symptoms of poor mental health, is mediated via cognitive processes.

1.2.11 Cognition as a hypothetical mediator

Cognitive processes may be a psychological mechanism by which dietary intake influences psychological health. Numerous studies have found an association between general cognitive status and symptoms of depression (Dotson et al., 2008). Moreover, cognitive deficits reduce daily functioning, quality of life, and increase the risk of mood disorders (O'Rourke et al., 2021; Withall et al., 2009; Xiang & An, 2015). Dietary intake may influence brain health, through cardio-metabolic impairment or nutrients with neuroprotective properties, and consequently cognition (Adan et al., 2019; Rogers, 2001; Townsend et al., 2023), which in turn could impact psychological health. Also, cognitive capability is known to increase positive emotions (Fredrickson & Joiner, 2002), while mild cognitive impairment reduces psychological wellbeing (Gates et al., 2014). Hence, some of the variability or individual differences in cognition may be related to dietary intake, that in turn, may impact psychological health. Additionally, cognitive impairment may reinforce unhealthful eating behaviour, creating a vicious cycle for psychological health. For instance, lower inhibitory control is associated with increased risk of overeating in individuals with negative emotional states, such as depression (Jasinska et al., 2012). Collectively, if subtle changes to cognition (e.g., memory and or executive control processes) occur as a result of dietary intake, then subsequent effects on psychological health may also be observed in general adult populations. Two aspects of cognition will now be discussed in more detail, this includes cognitive failures and inhibitory control.

Cognitive failures can be broadly defined as one's tendency to experience errors and slips in functioning (Broadbent et al., 1982; Wallace et al., 2002). Higher scores on the Cognitive Failures Questionnaire (CFQ) indicate more frequent errors and poorer cognitive functioning (Broadbent et al., 1982). Subjective CFQ scores in healthy older and middle-aged populations can be used to indicate early cognitive deterioration, prior to objective measures of cognitive decline (Langlois & Belleville, 2014; Papaliagkas et al., 2017). Notably, no previous research has explored cognitive failures as a hypothesised mechanism by which dietary intake impacts psychological health.

Cognitive failures encompass a range of cognitive errors in 'real world' planned thought and action (Carrigan & Barkus, 2016), such as walking into a room only to forget what you were looking for. There are concerns about task-based cognitive assessment regarding ecological validity (Burgess et al., 1998), therefore the CFQ offers a real-world measure of cognitive deficits. Assessing cognitive failures may be a relevant, sensitive, and ecologically valid approach to identifying increased vulnerability to cognitive and mental health difficulties (Wagle et al., 2004), which may act as a potential mediator in the relationship between dietary intake and psychological health. Furthermore, cognitive failures are a useful cognitive process to measure because multiple domains of cognition are captured, however, memory and attentional errors are the core facets of cognitive failures (Carrigan & Barkus, 2016). Self-reported cognitive failures scores have been associated with performance on laboratory-based cognitive tasks, including those that measure attentional networks, behavioural inhibition, and working memory (e.g., Berggren et al., 2011; Ishigami & Klein, 2009; McVay et al., 2009; Tipper & Baylis, 1987). Recently, it was identified that the CFQ represents a global measure of subjective cognitive difficulties, rather than errors in specific domains (Goodman et al., 2022), which may indicate early cognitive impairment (Clément et al., 2008).

Inhibitory control is a core executive function (higher-order cognitive process), it represents an individual's ability to regulate or inhibit prepotent responses, thereby facilitating attentional control, emotion regulation, and goal-directed decisions or actions, which have all been implicated in psychological health (Diamond, 2013). Inhibitory control may be another mechanistic pathway through which dietary intake impacts psychological health. Research highlights that poor inhibitory control is linked to overeating, emotional eating, and binge eating (Boswell et al., 2018; Houben et al., 2014). These eating behaviours are consistently related to poor diet quality and psychological health (Schag et al., 2013). Diets high in sugar and saturated fats have been associated with deficits in inhibitory control (Francis & Stevenson, 2011; Kanoski & Davidson, 2011), whereas nutrient-rich diets have been shown to enhance inhibitory processes (Cohen et al., 2016; Valls-Pedret et al., 2015). The Stop-signal task is a validated and widely used cognitive task that assesses inhibitory control, and is sensitive enough to measure interindividual variations and change over time in inhibitory control abilities (Verbruggen & Logan, 2009). The relationship between dietary intake and psychological health is a complex, a range of possible mechanisms through which dietary intake may influence psychological health exists (e.g., cognitive, emotional, and biological process), however, very little attention has been given to cognition as a psychological mechanism.

1.3 Methodological Gaps and Causation

Given the health, social and economic burden of impaired psychological health, there are calls for new preventative public health approaches (Jané-Llopis et al., 2011), with recent research suggesting that dietary intake is a potential modifiable lifestyle target for improving psychological health (Dharmayani et al., 2021; Glabska et al., 2020). Previous research has focussed on the link between diet patterns or diet quality and psychological health (Lai et al., 2014; O'Neil et al., 2014), however, further research that explores the direct and indirect relationships between nutrient-rich (F&V) and nutrient-poor (e.g., sweet, and savoury snacking) food consumption and psychological health will enable the identification of dietary targets for protecting mental health and enhancing psychological wellbeing. F&V intake needs to be studied in a more nuanced way, with fruit and vegetables being evaluated as separate dietary components to enable the analysis of their isolated impact. Furthermore, the quantity and frequency of intake should both be considered to understand how to maximise effects (Ocean et al., 2019). The unique contribution of FVI relative to other lifestyle factors needs to be scrutinised, through the consistent inclusion of covariates in analyses. Dietary intake is one component of overall lifestyle behaviours that may work in combination or alone to influence psychological health (Collins et al., 2022). Several lifestyle factors contribute to the development of poor psychological health, which are related to cognition and wellbeing (Cassidy et al., 2022; Davison et al., 2022). These include physical activity, smoking, and sleep duration (Wickham et al., 2020). To draw accurate conclusions regarding the unique impact of dietary intake, independent of lifestyle factors and individual differences, a range of participant characteristics must be assessed (e.g., exercise, general health rating, eating style, BMI, income, education, age). Furthermore, a wider range of aspects of psychological health outcomes, including symptoms of poor mental health and wellbeing gains should be considered within healthy adult populations (Quirk et al., 2013; Rooney et al., 2013). Thus, a more rigorous assessment of dietary intake, psychological wellbeing and mental health will extend the current knowledge base and inform causal inferences.

Research assessing the impact of dietary components on cognitive outcomes is limited, few studies have explored the relationship between snacking and cognitive performance with inconsistent results (Flueckiger et al., 2017; Miller et al., 2013), and the types of snacks were not always specified. However, evidence shows that foods high in saturated fat and sugar impair cognitive processes such as executive function and memory (Francis & Stevenson, 2011; 2013; Stevenson et al., 2020). Previous research has emphasised that a F&V-rich diet has been shown to be consistently protective of psychological health in older adults over time (Wu et al., 2021). Indeed, habitual nutrient-rich dietary intake may cumulatively protect cognitive and mental health as we age, hence the links found in older adults (Gehlich et al., 2019). Further prospective research evaluating the diet-cognition, diet-psychological health links in young adults is called for (Appleton et al., 2023; Francis et al., 2019). Older populations often include participants with comorbidities or cognitive decline, and this limits the generalisability to the general adult population as results may be conflated due to larger margins for change in cognition or psychological health (Adan et al., 2019; Byers et al., 2010; Prenderville et al., 2015). Thus, further research across the lifespan that focusses on healthy young to middle age will more robustly evaluate the relationship between dietary intake, cognition, and psychological health.

Importantly, the identification of the underlying mechanisms of the relationship between dietary intake and psychological health will inform both directionality and causality. Evidence indicates that dietary intake predicts cognitive function (Stevenson, 2017), and impaired cognition is related to a deterioration in psychological health (Gates et al., 2014; Knight et al, 2020). However, the psychological mechanism of cognitive processes as a hypothetical mediator in the relationship between dietary intake and psychological health requires investigation (Rao et al., 2008). Plausible biological mechanisms exist that may explain the relationship between FVI and psychological health, such as the physiological effects of antioxidants or the neurochemical effects of folate in the brain (Bottiglieri, 2005; Martone, 2016; Moylan et al., 2014). Epidemiological studies show that folate and other B vitamins are specific nutrients that may protect psychological health (Sanhueza et al., 2013), so certain types of vegetables may provide a specific source of nutrients that protect psychological health, of which folate may play a pivotal role. However, comparison of the effects of vegetables, known to contain high levels of folate, with folate supplementation is yet to be conducted. Overall, the research within this thesis will focus on replication and applying a uniform approach to dietary and a range of psychological health measurement, as recommended by reviews (Glabska et al., 2020; Rooney et al., 2013). To enable more robust conclusions to be drawn from the data, the research will consider a range of covariates when evaluating the relationship between dietary intake, cognition, and psychological health. Moreover, the research samples will focus on the recruitment of young to middle aged adults to expand the current literature. In addition, a novel evaluation of the psychological mechanism of cognitive processes as a hypothetical mediator will be assessed. Furthermore, the effects of acute folate consumption as a micronutrient with known biological function relevant to psychological health will be explored.

1.4 Thesis Aims

The aims of this thesis were to firstly assess the impact of vegetable, relative to fruit consumption, on psychological health, through a systematic review of prospective data. Secondly, to investigate the complex relationship between nutrient-rich FVI separately, nutrient-poor sweet and savoury snacks (and overall diet quality), and psychological health, within the general and young adult populations while accounting for covariates, both cross-sectionally, and over time also. Thirdly, to evaluate whether cognitive processes act as a mediator in this relationship on psychological health, including symptoms of depression, anxiety, stress, and positive psychological wellbeing. Finally, to experimentally explore the acute effect of folate (in supplement and food form) on mood, affect, cognition, and psychological health in healthy adults in controlled laboratory settings. To realise these aims, quantitative research methods were used to evaluate the associative relationships between dietary intake and psychological health, in addition to analysis over time, and laboratory-based experimental manipulations of intake. This thesis comprises five empirical chapters consisting of a systematic review, two cross-sectional studies, a prospective study, two pilot laboratory experiments, and an RCT. The samples involved the general UK adult population, young adults, university students and staff.

Chapter 2 The Effects of Vegetable Consumption on Psychological Health; A Systematic Literature Review of Prospective Research in Healthy Adults

This chapter was published in The American Journal of Clinical Nutrition, only slight adjustments have been made to ensure cohesion within the thesis.

Tuck, N. J., Farrow, C., & Thomas, J. M. (2019). Assessing the effects of vegetable consumption on the psychological health of healthy adults: a systematic review of prospective research. The American Journal of Clinical Nutrition, 110(1), 196-211.
https://doi.org/10.1093/ajcn/nqz080

While the previous narrative review provides a synopsis of the literature, a more exhaustive review of the independent effects of F&V (separately) on several aspects of psychological health is required. This includes symptoms of depression, anxiety, stress, distress, and positive psychological wellbeing. To better understand the causal relationship between vegetable consumption and these outcomes, only prospective and experimental evidence will be synthesised for evaluation. The findings will inform the design and focus of the subsequent studies within this thesis. This chapter addresses the first aim of the thesis to assess the impact of vegetable, relative to fruit consumption, on psychological health, through a systematic review of prospective data. This systematic review aimed to synthesize and evaluate research investigating the effects of vegetable consumption on mental health and psychological wellbeing in non-clinical, healthy adult populations. Ten eligible studies were identified with a total sample size of n= 33,645, that measured vegetable intake separately from fruit, or combined this with fruit intake. Where studies explored the independent effects of fruit and vegetable consumption on psychological health (n=3), two reported a preferential effect of vegetables (vs. fruit) on psychological wellbeing, whereas one reported a superior effect of fruit intake on odds reduction of symptoms of depression. More broadly, there was evidence that consuming the recommended amount of F&V (and exceeding this), was associated with increased psychological wellbeing. However, the effects of F&V consumption on mental health symptoms were inconsistent.

2.1 Introduction

Recent evidence suggests that nutrition may be an important resilience factor against mental disorders, given that diet quality is consistently associated with mental health across a range of age groups (Akbaraly et al., 2009; Gomes et al., 2018; Jacka et al., 2010, 2012; Marx et al., 2017; Sánchez-Villegas et al., 2015). However, if changes to diet can induce positive changes in mental health, what foods or food groups should be targeted? A growing body of evidence suggests that there is a link between fruit and vegetable (F&V) consumption and psychological health. For instance, results from a cross-sectional study using participants' health records, indicated that vegetable consumption is associated with reduced risk of depression diagnosis by a physician (Meyer et al., 2013). More broadly, greater F&V consumption has been consistently associated with lower odds of psychological distress and major depressive disorder (McMartin et al., 2013; Nguyen et al., 2017; Ribeiro et al., 2017). Previous research has also demonstrated an association between habitual F&V consumption and psychological wellbeing (Hong & Peltzer, 2017; Lesani et al., 2016; Mujcic & Oswald, 2016; Peltzer & Pengpid, 2017). Further, a series of cross-sectional studies have reported that high F&V intake is also associated with positive psychological health outcomes (Blanchflower et al., 2013).

Given the difficulty in raising the consumption of F&V by the general population, it is worth considering whether F&V are equally associated with, and exert effects on, mental health and psychological wellbeing. A previous systematic review reported that F&V consumption are independently protective against depression (Saghafian et al., 2018). Suboptimal intake of micronutrients (folate and iron) found in leafy green vegetables is associated with mental health being perceived as poor, and symptoms of depression (Davison et al., 2017; Gilbody et al., 2007a). Further, a recent meta-analysis identified the consumption of vegetables, but not fruit, was associated with depression risk (Molendijk et al., 2018). Thus, it is plausible that vegetable consumption may provide additional benefits to psychological health, than fruit consumption.

The aim of this systematic review was to identify, appraise and evaluate evidence from studies with prospective data investigating the effects of vegetable intake on the mental health and psychological wellbeing of healthy adults. There is a need for research to quantify and focus on wellbeing gains in non-clinical populations in order to go beyond the reversal of ill-being. The synthesis of the research aims to elucidate what is currently known, and what future research is required, to understand the influence of vegetable intake (independent of fruit) on mental health and psychological wellbeing. It was hypothesised that vegetable intake would have a superior influence on psychological health (vs. fruit). To identify the extent to which vegetable intake has an effect on these outcomes, the findings from studies where fruit and vegetable intake were each measured separately, and from studies where fruit and vegetable intake were measured as a combined construct, were examined, and compared.

2.2 Method

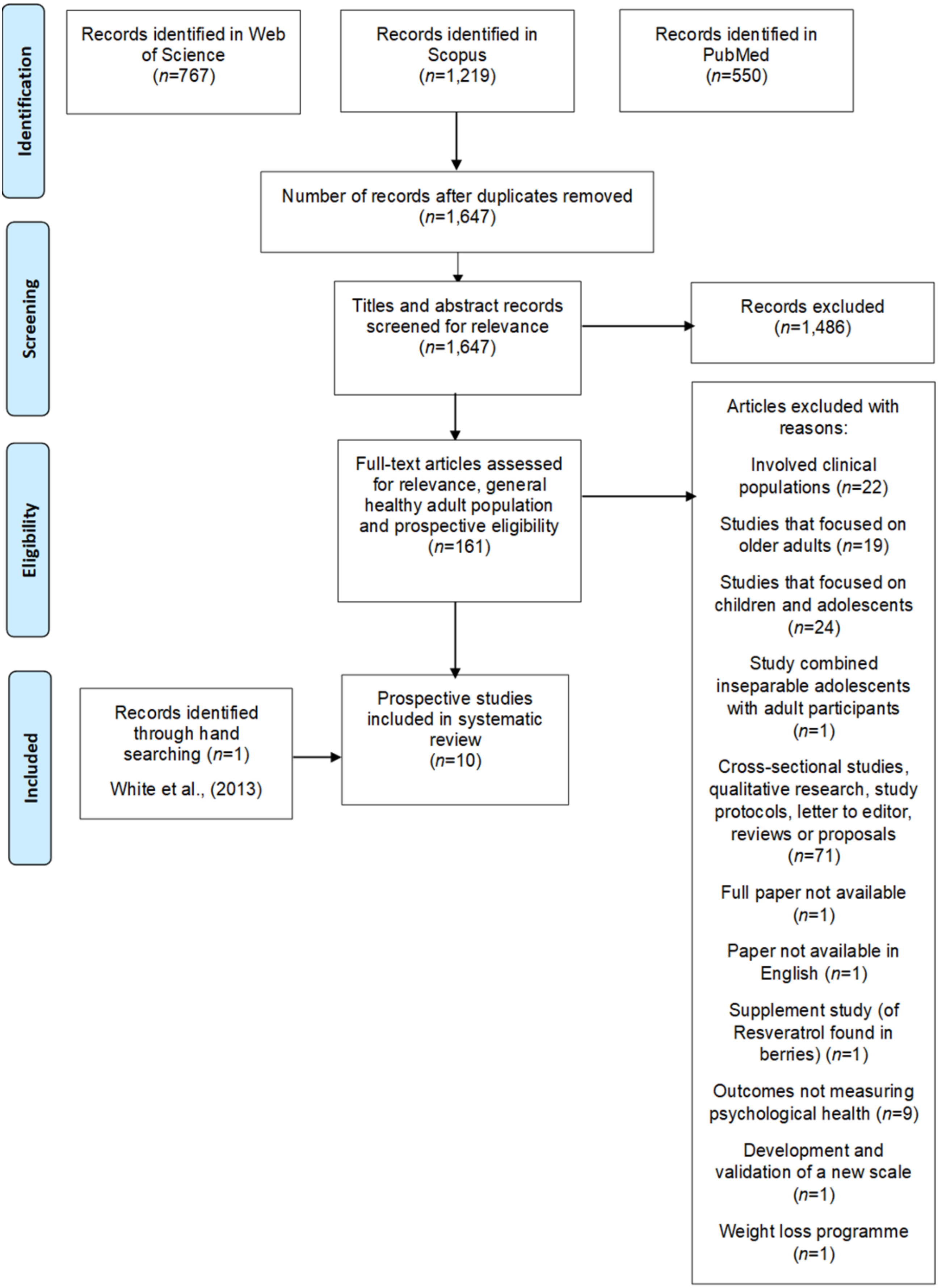
2.2.1 Review registration

The review was registered with PROSPERO International and can be accessed online using the web address https://www.crd.york.ac.uk/PROSPERO and entering the following registration ID: CRD42017072880.

2.2.2 Literature search

The search strategy used three medical, health, psychiatric and social sciences databases. The first, Web of Science (all databases), is a research focused search engine that allows the user to search a number of subscribed databases in tandem. The subscribed databases available to Web of Science were: Web of Science Core Collection, BIOSIS Citation Index, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index and SciELO Citation Index. The second database utilised was Scopus and the third was PubMed. The search terms included vegetable* combined with psychological* and health. The search involved the topic (title, abstract and keyword) approach. All references were downloaded into the computerised bibliographic program Endnote (Endnote citation) that facilitated the handling of the large numbers of publications obtained. The searches were last implemented on the 1st February 2019. The database searches yielded 2536 citations, with one additional article identified through hand searching the reference lists of relevant studies. After removal of duplicates, 1647 citations remained. Screening of titles or abstracts for relevance resulted in 161 papers being identified for full-text assessment. Additionally, one hand searched paper was identified. Of these, 152 articles were excluded; the outcome of the searches and reasons for exclusion are detailed in the PRISMA flow diagram (Figure 2.1). Ten full-text articles were included in the final analysis.

Figure 2.1 PRISMA flow diagram of the search and study selection.



2.2.3 Study selection

This review included studies with healthy adult populations and the majority of participants were aged between 16 and 62 years of age. The healthy adult population were defined as participants without clinical characteristics and who were not referred to as patients. Two community sample studies represented a wide range of ages. The first study involved participants aged 16 years and older, however, no upper age cut off was reported (Blank et al., 2007). The second study involved participants aged 16 years and older with some participants being 65-74 years old (8%) and 75+ years old (7%) (Johnson et al., 2017). Studies that focused on older adults were excluded as the increased prevalence of health complications for older individuals might alter the influence of vegetable intake on psychological health. Also, studies involving children and adolescents (<16 years of age) were excluded because analysis of psychological health is difficult in this population. In addition, recommended F&V intake tends to be age-dependent, therefore, this review focused on the adult population. Studies involving clinical populations were also excluded because physiological changes due to health complications might confound the results. For example, studies involving primary care or cancer patients were excluded. There were no case-control design studies where healthy and clinical samples were compared that met the inclusion criteria.

To provide insight into the temporal association between vegetable intake and psychological health, only studies with prospective or experimental data were included. Studies that met the inclusion criteria were observational or epidemiological designs, including before and after, macro-longitudinal or micro-longitudinal studies, a randomised-controlled trial and cohort studies. Cross-sectional studies, qualitative research, study protocols, reviews or proposals were excluded. Studies were included regardless of the use of a comparison group. Papers were excluded on the basis of their availability (access to the full paper was required) and language (only those written in English were included). This review included studies that reported intake of vegetables independently of fruit, but also included those that combined vegetable with fruit intake. This was in order to compare the effect of vegetable consumption against combined F&V consumption. The review included intervention studies or those that measured self-reported vegetable consumption. The review did not include studies focussed on dietary patterns unless they were referred to specifically as a vegetable dietary pattern. This review only considered studies that evaluated outcome measures related to mental health or psychological wellbeing, however, given the large number of measures in these respective fields, individual measures were not prespecified as specific inclusion or exclusion criteria. Risk of suicide as an outcome was excluded because this was considered an extreme end point of mental health.

2.2.4 Data extraction

The review was undertaken using PRISMA guidelines (Moher et al., 2009). The details from all studies were organised by one review author (NT) and checked by the additional review authors

(JT & CF). The data extracted included specific details about the study aims, duration, sample size, age, population, assessment or implementation of F&V intake, psychological health assessment tool, adjustment for confounders and outcomes of significance to the review question (as presented in Table 2.1). Due to the early nature of the research area, the limited number of studies available, and high heterogeneity between study methodologies, the risk of bias was not assessed, and studies were not synthesized for meta-analysis.

Table 2.1 Study information by study duration, sample, measures, and results.

Author, date of publication, country	Aim	Study duration	No. of participants, sex (%), age at recruitment, mean age (SD) and follow-up statistics	Population description	F&V intake measure	Combined or independent measure of F&V intake used	Psychological health assessment measure	Adjustment for confounders	Main findings	Statistical analysis & results	Conclusions
Blank (2007) UK	To explore the association between change in diet and physical activity rates and self-reported mental and overall health of residents living in deprived English communities.	Two years from 2002 to 2004.	11,377 16 years and older, mean age/ upper limit not reported, or sex % or SD *n*=10,419 during 2004 follow-up.	Participants were from community-based areas participating in the New Deal for Communities (a major government funded community development initiative). Sample included 958 comparator 'stayers'.	The quality of diet was rated from 1-5 based on the portions of F&V consumed per day.	Combined F&V intake, not analysed separately.	SF-36 mental health score. A combined measure of 5 items.	Gender, age, education, tenure (owner occupied), ethnicity, study area of residence (NDC or comparator), workless household 2002, SF-36 mental health score 2002, physical activity score 2002 and diet score 2002.	Improved diet and mental health associations were stronger for the more positive items: calm and peacefulness (adjusted odds ratio=1.36) and happiness (adjusted odds ratio=1.49). Improved diet was also related to reductions in nervousness (adjusted odds ratio=1.15).	Paired comparisons showed that improved self-rated mental health is associated with a better diet in 2004 compared to 2002. A worsening of self-reported mental health is associated with worse diet. These associations only reach statistical significance for those with the greatest improvement in their diet (2.71, $p<0.01$).	Significant associations were found between 'large' positive behaviour change in diet (increased F&V intake) and mental health.
Conner (2015) New Zealand	To test whether F&V consumption is related to eudaimonic wellbeing and eudaimonic behaviours.	Thirteen days in 2013.	405 young adults (66.7% female), 17-25 years old, mean age was 19.85 (SD=1.62). *n*=392 (6 dropped out; 7 completed fewer than 7 diary records).	Undergraduate student participants in the 2013 wave of the Daily Life Study, a large interdisciplinary study of health and emotional experiences of young adults.	An online daily survey using questions modified from the New Zealand Nutrition Survey (1997) was used to quantify F&V portions consumed that day (fresh, frozen, or canned vegetables not including juice or chips).	Analysed separately. Number of portions consumed per day were reported independently for F&V.	Daily eudaimonic wellbeing was measured using an adaptation of the Flourishing Scale (2009). Daily affect was measured through ratings of 9 adjectives of positive affect (PA) and 9 adjectives of negative affect (NA).	Gender, previous day wellbeing and previous day food consumption. Weekend effects were controlled in all lagged analyses.	Vegetable consumption predicted increases in daily eudaimonic wellbeing ($p<0.001$) after controlling for daily changes in PA. On days when participants ate more F&V they reported greater eudaimonic wellbeing and positive affect than on days when they ate less F&V. Consumption of vegetables, but not fruit, was associated with lower negative affect.	Multilevel modelling with a Hierarchical Linear Modelling software program. The model allowed analysis of food consumption and wellbeing covariance within an individual over time, controlling for weekday versus weekend effects. Next-day lagged analyses were conducted for all significant within-person associations between the food and wellbeing measures to test if consumption predicted changes in next-day wellbeing.	F&V additively contributed to positive changes in eudaimonic states, however vegetables played a stronger role. The within-person design shows that wellbeing fluctuated as a function of F&V consumption within the same person over time. It cannot be determined whether F&V caused the day-to-day changes to wellbeing.
Conner (2017) New Zealand	To test the psychological benefits of a preregistered clinical intervention to increase F&V consumption in young adults.	Fourteen days in 2015.	*n*=171 67% were female; 18-25 years old, mean age was 19.43 (SD=1.45).	Young adults from the undergraduate student population or members of a student employment agency.	Self-reported F&V intake via smart phone surveys. RCT design involving 3 conditions: provision of F&V, text message reminders to increase intake and a voucher to purchase F&V, and a diet as usual control. Fasting blood samples for Vitamin C and carotenoid levels were taken.	Combined measure and provision of F&V (including more fruit, carrots were the vegetables).	Centre for Epidemiologic Studies Depression Scale (CESD;1977), an Anxiety Subscale of the HADS (1983), 3-item measures of negative and positive mood, a 4-item vitality scale, 3-item Flourishing Scale and a psychological expectations survey.	Ethnicity and psychological expectations. Conditions were balanced in terms of gender, age and BMI.	Provision of F&V to consume but not reminders improved psychological wellbeing over the fourteen-day period. Only participants given the two weeks worth of F&V showed improvements in feelings of vitality, flourishing and motivation. Despite relatively small changes in F&V consumption in the provision condition (+1.2 portions from baseline).	ANCOVA with condition as the between-subject variable, time as the within-subject variable. Hierachical Linear growth curves to assess greater improvements in mood and wellbeing over time relative to the control condition. Neither vitamin C and carotenoids nor psychological expectancies mediated the F&V psychological benefits of consumption.	The effects of the intervention were prominent across measures of psychological wellbeing but not ill-being (depression, anxiety or negative mood).

Author, date of publication, country	Aim	Study duration	No. of participants, sex (%), age at recruitment, mean age, SD and follow-up statistics	Population description	F&V intake measure	Combined or independent measure of F&V intake	Psychological health assessment measure	Adjustment for confounders	Main findings	Statistical analysis & results	Conclusions
Johnson (2017) UK	To examine changes in psychological wellbeing associated with a healthy lifestyle programme.	Twelve-week intervention and three months of follow up between April 2011 and April 2012. Data was collected at three time points: baseline, completion at 12 weeks and follow-up.	307 81% were female. All ages were represented from 16 years to 75+ years. 25% completed follow-up three months after the intervention *n*=121.	Community sample in the West Midlands recruited through media publicity, GP referral, posters in the community centres etc.	Self-reported using items 'on a typical day, how much fruit and vegetables do you eat (dried, fresh, frozen, or tinned, one portion is the same as a handful). Responses were predetermined as 0 to 5+ portions.	Combined portions of F&V, not analysed separately.	WEMWBS that covers affective and psychological functioning constructs of wellbeing.	Models adjusted for gender, age, physical activity and walking.	Change in F&V intake (independent from physical activity) explained some, but not all of the change in psychological wellbeing associated with the intervention. F&V intake explained greater variance than physical activity.	Inferential analysis was conducted to identify change in WEMWBS over time, statistical significance and effect size of change.	Healthy lifestyle interventions could improve psychological wellbeing, and improvements can be partly explained by increased F&V intake. Improvements in wellbeing were sustained from completion to the 3-month follow-up.
Kingsbury (2015) Canada	To examine the associations between F&V consumption and depression across multiple time points and to test the reverse model: the association between depression at each time point and subsequent fruit and vegetable intake.	Every 2 years from 2002/2003 to 2010/2011.	*n*=8,353 47.21% male, 18 years and over, mean age 44.16 (SD=18.41).	Participants were from the National Population Health longitudinal survey.	Daily consumption frequency of fruits, green salad, carrots and other vegetables was assessed using questions from the BRFSS questionnaire. Juice and potatoes were not included.	Combined F&V intake, not analysed separately.	Major depression was measured using the Composite International Diagnostic Interview (CIDI-SF). Distress was measured using the Kessler Psychological Distress scale (2002).	Age, gender, history of major depression, education, income, chronic illness, binge drinking/ smoking status, obesity and physical activity.	Greater F&V consumption during a given cycle was associated with lower risk of depression and distress scores at the next cycle (when the model was adjusted for age, gender, history of depression, education, SES, chronic illness).	Generalised estimating equations regression models were used to model the associations between F&V consumption at each cycle and depression status/distress score at the next cycle. When the model for depression was adjusted for obesity, the relationship was no longer significant. When the model for distress scores was adjusted for social support, smoking and physical activity, the relationship was no longer significant.	Using a time-sensitive approach by adjusting for confounders at multiple time points, the relationship between F&V consumption and indicators of mental health were attenuated. Other health-related behaviours may have a more important impact on symptoms of depression than consumption of F&V.
Lee (2016) Korea	This study explored the effects of switching to a vegetarian diet (rich in F&V) on stress reduction, nutritional status and bowel habits.	Twelve week vegetable-focused diet adopted (April to July 2013).	*n*=14 teachers, 3 male, 11 female, age not reported. The study also included 26 adolescent students but their data was not included in this review.	School-based study.	The school dietician provided vegetable-based meals. The diet included vegetables (25.3%) and fruit (19.5%). Educational sessions were conducted to maintain the vegetable-focused diet habits at home outside of school times.	Self-reported F&V intake was not gathered.	Stress was measured using the Perceived Stress Scale (1983)	None stated.	The stress levels of teachers were substantially reduced to 19.57 (±9.68 points) from 22.64 (±10.73). Blood lipid profiles were significantly improved. The level of vitamin B12 increased significantly.	Paired t-tests were used to analyse the changes to stress levels at week 0 to week 12.	An improvement in stress levels coincided with the adoption of a vegetable-focused diet. Stress levels were significantly reduced after 12 weeks. Nutritional status measured by blood profiles and bowel conditions also improved.

Author, date of publication, country	Aim	Study duration	No. of participants, sex (%), age at recruitment, mean age, SD and follow-up statistics	Population description	F&V intake measure	Combined or independent measure of F&V intake	Psychological health assessment measure	Adjustment for confounders	Main findings	Statistical analysis & results	Conclusions
Mihrshahi (2015) Australia	To evaluate the differential effects of F&V intake in relation to depression and the temporal sequence of the possible association.	Two-three year intervals, over a 6 year period between the years of 2004-2010. Prospective cohort data was used from survey 4 in 2004, survey 5 in 2007 and survey 6 in 2010.	*n*=6,271 Mid-aged women, mean age was 55.45 (SD=1.45). 45-50 years old at baseline.	Mid-aged cohort of women participating in the Australian Longitudinal Study on Women's health (ALSWH).	F&V intake was quantified by the responses to two questions regarding quantity of portions consumed per day.	Number of portions consumed per day were reported and analysed independently for F&V.	Incidence of depression was defined by the presence of symptoms of depression measured by the 10-item CESD Scale (1977) at a survey and the absence of depression at the survey immediately before it. Prevalence of depression was defined as presence of depression at that survey, irrespective of the preceding survey.	BMI, educational attainment, marital status, smoking and alcohol status, physical activity, comorbidities, energy and specific nutrient intake from a FFQ.	Eating the recommended amount of fruit was associated with reduced odds for symptoms of depression. There was no clear effect of eating the recommended amount of vegetables per day on incidence of symptoms of depression. There was a cross-sectional association between vegetable intake and prevalence of symptoms of depression at higher levels of intake (equal to or greater than 5 portions/day).	The association between F&V intake and prevalence of symptoms of depression over three surveys was modelled using logistic regression with generalised estimating equations (GEE) to account for repeated measures. Longitudinal GEE modelling to determine the effects of FV intake on the incidence of depression.	There seems to be a differential effect of fruit and vegetable intake, which may not be additive, with fruit intake being more important than vegetables for symptoms of depression.
Rienks (2013) Australia	To investigate the association between dietary patterns and prevalence and incidence of symptoms of depression.	Three years from 2001 to 2004. Prospective cohort data was used from survey 3 and survey 4.	*n*=7,588 sample for longitudinal analysis (no missing data). Mid-aged women aged between 45-50 years at baseline, mean age was 52.5 (SD=1.5).	Mid-aged cohort of women participating in the Australian Longitudinal Study on Women's health (ALSWH).	Dietary intake was assessed at survey 3 using a FFQ developed for use in Australian adults. Participants obtained a score for six dietary patterns, including a 'cooked vegetables' pattern (cauliflower, cabbage, sprouts, broccoli and green beans).	Scores for a fruit dietary pattern and a vegetable dietary pattern were explored independently.	Symptoms of depression were measured using the 10-item CESD scale (1977) at survey 3 and 4.	Energy intake, smoking, physical activity, ability to manage on available income, occupation status, education level, marital status, mean stress score and BMI.	No significant associations were found for the fruit or vegetable dietary pattern. Only an inverse relationship for the 'Mediterranean-style' dietary pattern was found and not attenuated after adjusting for confounders and symptoms of depression at survey 3.	Factor analysis to identify dietary patterns from the FFQ, longitudinal analysis to investigate symptoms of depression at survey 4. Multiple logistic regressions to estimate the association between symptoms of depression at survey 4 and each of the six dietary patterns, while adjusting for other confounders.	The consumption of a 'Mediterranean-style' dietary pattern (garlic, peppers, mushrooms, salad greens, pasta and red wine) by mid-aged women may have a protective influence against the onset of symptoms of depression 3 years later.
Sutliffe (2018) USA	To determine the impact and effectiveness of a micronutrient-dense, plant-rich dietary intervention on employee wellbeing when administered at the worksite.	Six-week nutrition intervention.	*n*=35 (pilot study) 91.4% female, mean age was 42.57 years old (range: 24-61 years).	University employees with a BMI ≥ 25.	Micronutrient-dense, plant-rich diet (Nutritarian diet) was implemented. This was a vegetable-based diet that emphasized daily consumption of greens, beans and legumes, variety of other vegetables, fresh or frozen fruits, nuts, seeds and wholegrains. Multivitamins were also encouraged.	Self-reported F&V intake was not gathered.	Symptoms of depression were measured using the Beck Depression Inventory-II and The Quality of Life Index Generic Version-III was used to measure quality of life in terms of satisfaction with life.	Dietary compliance, attendance and physical activity were explored in relation to change in outcome variables. To rule out physical activity as a confounder, participants wore an activity tracker during a seven day portion of the intervention.	There was a significant change in overall Quality of Life Index after the intervention. Significant changes were observed in the Health and Psychological subscales. There was a significant change/ reduction in mean depression scores after the intervention. Physical activity was not correlated with changes in the outcome measures.	Paired sample t-tests and a Wilcoxon Signed Ranks test were used to determine significant changes in the outcome measures before and after the intervention.	Improvements in overall wellbeing and mood may be due to the micronutrient-dense, plant-rich diet as well as a subsequent reduction in refined foods and animal products.
White (2013) New Zealand	To explore the relationships between daily negative and positive affective experiences and food consumption in a naturalistic setting.	Twenty-one consecutive days between April 2008 and August 2009.	*n*=281 55.4% were female; mean age was 19.9 (SD=1.2).	Healthy, young adults from the undergraduate student population.	Internet-based daily diary including five food consumption questions modified from standard questions in the New Zealand Nutrition Survey (1997).	Number of F&V portions consumed per day were reported and analysed independently.	Ratings of 18 adjectives, 9 negative affect adjectives and 9 positive affect adjectives. Averages for each were calculated.	BMI and gender as moderator variables.	On days when individuals experienced higher positive affect, they consumed 0.112 more portions of fruit (p=.002) and 0.147 more portions of vegetables (p<.001). These associations exceeded the adjusted significance threshold of p<0.005.	Multilevel modelling with a Hierarchical Linear Modelling software program to analyse how affect and food consumption co-varied within a given individual over time. Next-day analyses were conducted for all significant affect-eating associations to explore causality.	On days when individuals consumed more portions of fruit and/or vegetables, they reported experiencing greater positive affect the following day, while controlling for current-day intake. Reverse causality was not significant.

Note: SD, Standard deviation; F&V, fruit and vegetables; SF-36, Short Form Health Survey ; NDC, New Deal for Communities, a major government funded community development initiative; PA, positive affect; NA, negative affect; RCT, Randomised Controlled Trial; CESD, Centre for Epidemiologic Studies Depression Scale, BMI, Body Mass Index; HADS, Hospital Anxiety Depression Scale, ANCOVA, Analysis of Covariance; WEMWBS, Warwick-Edinburgh Mental Wellbeing Scale; BRFSS, Behavioral Risk Factor Surveillance System of the USA Centers for Disease Control and Prevention; SES, socioeconomic status; FFQ, Food Frequency Questionnaire.

2.2.5 Quality assessment

One of the authors (NT) and an independent researcher, separately coded the quality of the studies using an adaptation of Lievense et al.'s (2002) scoring system that includes the questions applicable to prospective studies. Where scores differed, both coders discussed the items to reach full agreement. This quality checklist has been used in systematic reviews of diet and mental health in children and adolescents (O'Neil et al., 2014) and the association between diet patterns and depression in adults (Quirk et al., 2013). Therefore, it was deemed a suitable assessment of methodological quality for this systematic review. The methodological quality assessment of the included studies involved evaluation of the use of validated measures, outcome reproducibly, follow up time, withdrawals and variables adjusted for during data analysis. Each of the 11 criteria items were scored as follows: positive (1), negative (0), or unclear (?) with 100% representing a maximum possible score. Studies were defined as high quality if the total quality score was above the mean of all quality scores (69%). See Table 2.2 for the quality scores of the studies organised in descending order.

Table 2.2 Methodological quality of included studies.

Study	Quality rating	Score	Percentage
Conner (2017)	High	9/11	82%
Rienks (2013)	High	9/11	82%
Johnson (2017)	High	8/11	73%
Mihrshahi (2015)	High	8/11	73%
Blank (2007)	High	7/10	70%
Kingsbury (2015)	High	7/10	70%
Lee (2016)	Low	7/11	64%
Conner (2015)	Low	6/10	60%
Sutliffe (2018)	Low	6/10	60%
White (2013)	Low	6/11	55%

2.3 Results

A total of ten studies were identified, one of which was hand-searched (White et al., 2013) with a total sample size of n= 33,645. One study was a randomised controlled trial (RCT) involving an intervention to increase F&V consumption in young adults (Conner et al., 2017). Three of the studies were prospective cohort designs which analysed longitudinal health survey data retrospectively (Kingsbury et al., 2015; Mihrshahi et al., 2015; Rienks et al., 2013). Two of the studies were micro-longitudinal studies involving daily diary surveys within the young adult population (Conner et al., 2015; White et al., 2013). These studies explored fluctuations in wellbeing as a function of daily F&V consumption by the same person over time (correlational analysis on a day-to-day basis). Two studies evaluated outcomes associated with community lifestyle change projects; the first examined a major government-funded community development initiative by analysing longitudinal data (Blank et al., 2007), the second involved a twelve-week healthy lifestyle intervention and was a before and after study (Johnson et al., 2017). Two before and after studies changed eating behaviour by implementing a vegetable-focused diet (Lee et al., 2016) and a micronutrient-dense, plant rich diet and measured the outcomes (Sutliffe et al., 2018). The majority of studies were published after 2013 (n = 9, 90%).

Sample sizes ranged from fourteen adult participants (Lee et al., 2016) to a cohort study with 10,419 participants (Blank et al., 2007). Two of the reviewed studies examined a middle-aged cohort comprising women only (Mihrshahi et al., 2015; Rienks et al., 2013), seven studies were mixed with regards to gender and one study did not report the distribution (Blank et al., 2007). Three of the studies were conducted in New Zealand (White et al., 2013; Conner et al., 2017; Rienks et al., 2013) and two involved data from the Australian Longitudinal Study on Women's Health (Mihrshahi et al., 2015; Rienks et al., 2013). Additionally, two studies involved UK based community samples (Blank et al., 2007, Johnson et al., 2017). The remaining studies were undertaken in Canada (Kingsbury et al., 2016), the USA (Sutliffe et al., 2018) and Korea (Lee et al., 2016). Two studies were population-based (Johnson et al., 2017, Kingsbury et al., 2016), there were some specific population groups investigated including: teachers (Lee et al., 2016), university employees with a BMI ≥ 25 (Sutliffe et al., 2018), university students (White et al., 2013; Conner et al., 2017; Conner et al., 2015), residents living in deprived English communities (Blank et al., 2007) and middle-aged women (Mihrshahi et al., 2015; Rienks et al., 2013).

2.3.1 Assessment of fruit and vegetable intake

Three studies evaluated the effects of fruit and vegetables independently, one on measures of psychological wellbeing (Conner et al., 2015), the second on symptoms of depression (Mihrshahi et al., 2015) and the third on daily experiences of negative and positive affect (White et al., 2013). One study analysed the outcomes of dietary patterns including a 'cooked vegetables' dietary pattern, which included intake of cauliflower, cabbage, Brussels sprouts, broccoli and green beans (Rienks et al., 2013). The RCT study (Conner et al., 2017) assigned participants to a F&V intervention condition in which two daily portions of fresh F&V (carrots, apples, kiwi-fruit, or oranges) were provided that supplemented their normal diet. The comparison condition involved text-message reminders and a voucher to purchase F&V, both aimed to increase consumption. The control group maintained their normal diet. Another study (Lee et al., 2016) explored the effects of adopting a vegetarian diet, where: the supply of vegetable-focused foods was increased; educational sessions on vegetable-related nutrition were provided, and the consumption of processed foods was restrained to maximize the effectiveness of the vegetarian diet. The final study (Sutliffe et al., 2018) involved the implementation of a vegetable-based, micronutrient-dense, plant rich diet (referred to as the Nutritarian diet). Participants were also encouraged to minimize the consumption of processed or refined foods and limit the consumption of animal products (due to the low intake of animal products a multi-vitamin was also encouraged). Both of these vegetable-based dietary interventions (Lee et al., 2016, Sutliffe et al., 2018) did not include the assessment or reporting of vegetable consumption or a control, comparison group. However, blood profiles were analysed in the vegetarian diet study (Lee et al., 2016) before and after for mineral contents of zinc, iron, B12, calcium and magnesium as biological proxies of food intake.

To assess F&V intake a variety of techniques were employed across studies, including: online daily diaries (White et al., 2013, Conner et al., 2015) quantifying the portions of vegetables consumed using questions modified from the New Zealand Nutrition Survey (Parnell et al., 2001); surveys accessed via smartphones (Conner et al., 2017); food frequency questionnaires (Rienks et al., 2013); self-reported items of typical daily F&V intake (Johnson et al., 2017); independent questions of how many portions or pieces of vegetables and fruits were consumed per day (Mihrshahi et al., 2015); diet quality scores using one to five portions of F&V consumed per day as the indicator (Blank et al., 2007); and food frequency questions (Kingsbury et al., 2016) taken from the Behavioral Risk Factor Surveillance System of the USA Centers (2015). The time frame for these F&V intake measures were for habitual daily intake as portions, with the exception of one study (Rienks et al., 2013) that used frequencies of foods consumed over the last twelve months to calculate a 'cooked vegetables' dietary pattern score measured twice with a three-year gap between measurements, and two studies that did not measure self-reported F&V intake (Lee et al., 2016, Sutliffe et al., 2018). The studies that used online daily diaries or surveys accessed via smartphones required participants to recall daily F&V consumption for a duration of twenty-one days (White et al., 2013), thirteen days (Conner et al., 2015) or fourteen days (Conner et al., 2017). Further studies assessed F&V consumption twice, before and after a twelve-week lifestyle intervention (Johnson et al., 2017), on two occasions two years apart (Blank et al., 2007) and three times at two-year intervals (Mihrshahi et al., 2014). One study (Kingsbury et al., 2016) assessed F&V intake every two years between

2002/2003 to 2010/2011 and the sample included participants with at least one assessment of F&V intake between 2002/2003 and 2010/2011.

2.3.2 Assessment of mental health

A wide range of measures were used to examine mental health; the most common aspect measured was symptoms of depression. Three studies (Conner et al., 2017; Mihrshahi et al., 2015; Rienks et al., 2013) used the 10-item Centre for Epidemiologic Studies Depression Scale (39) to assess symptoms of depression. One study (Conner et al., 2017) also explored anxiety symptoms using the 7-item Hospital Anxiety and Depression sub-scale (Zigmond & Snaith, 1983). Another study (Kingsbury et al., 2016) explored depression status and major depression incidence using the Composite International Diagnostic Interview Short Form (Kessler et al., 1998), supplemented with the Kessler Psychological Distress scale (Kessler et al., 2002), which assesses the frequency of six non-specific symptoms of anxiety and depression. Another study (Sutliffe et al., 2018) measured symptoms of depression using the Beck Depression Inventory-II (Beck et al., 1996). One study (Lee et al., 2016) used the Perceived Stress Questionnaire (Cohen et al., 1983). Another study examined a global indication of mental health (Blank et al., 2007) by using the mental health items from the Medical Outcomes Study Questionnaire Short Form 36 Health Survey (Ware & Sherbourne, 1992) to quantify a self-rated mental health score.

2.3.3 Assessment of psychological wellbeing

Three studies applied specific measures of psychological wellbeing. One study (Johnson et al., 2017) included the Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS) (Tennant et al., 2007) which identifies affective and psychological functioning constructs of positive mental wellbeing. Another study (Conner et al., 2015) used the Flourishing Scale (Diener et al., 2009), which measures eudaimonic wellbeing and includes feelings of engagement, purpose and meaning in life. Eudaimonic wellbeing focuses on meaning and self-realization and defines wellbeing in terms of the degree to which a person is fully functioning as their authentic self (Ryan & Deci, 2001). This study also measured daily affect through ratings of negative and positive affect states (in the form of adjectives) based on the circumplex model of emotion (Russell & Barrett, 1999). Another study (Conner et al., 2017) explored psychological wellbeing using a shortened 3-item version of the Flourishing Scale (Diener et al., 2009). This was supplemented with measures of behaviours related to flourishing using the first item of the Curiousity and Exploration inventory (Kashdan et al., 2009), followed with a second item that measured creativity and a third that measured perceived motivation. These scores were combined to form an index of daily flourishing behaviours. Finally, one study (Sutliffe et al., 2018) used The Quality-of-Life Index Generic

Version-III (Ferrans & Powers, 1985) to measure quality of life in terms of satisfaction with life.

2.3.4 Symptoms of depression

One study (Mihrshahi et al., 2015) (quality score=73%) reported that eating the recommended amount of vegetables per day failed to prospectively predict the incidence of depression symptoms six years later. However, fruit consumption was significantly associated with reduced odds of symptoms in women who ate >2 portions of fruit a day, compared to those who consumed fewer. These findings do not support the hypothesis that vegetable consumption has a superior influence on mental health, nevertheless, they do support the notion that F&V exert different effects on mental health.

On the contrary, the RCT study (Conner et al., 2017) (quality score=82%) showed that a fourteen-day intervention involving the provision of mostly fruit (carrots, apples, kiwi-fruit/oranges) did not reduce symptoms of depression or anxiety relative to the control group. Even though daily reported F&V intake was significantly higher compared to the participants' baseline and the control group. Reductions in symptoms of depression associated with fruit intake may only be observed over a much longer period of time such as six years, as observed in the longitudinal study (Mihrshahi et al., 2015). Precise timescales, frequencies or quantities of vegetable consumption may be required to observe the benefits on symptoms of depression, but this cannot be explored without further data.

The dietary patterns study including a 'cooked vegetables' pattern (Rienks et al., 2013) (quality score=82%) showed that after adjusting for sociodemographic and lifestyle factors, only the 'Mediterranean-style' dietary pattern was found to result in lower odds of reporting symptoms of depression by 17% three years later. Participants in the highest intake quintile of the 'Mediterranean-style' pattern were less likely to report subsequent symptoms of depression by more than a third (odds ratio 0.63, 95% confidence interval 0.47-0.85) compared with participants in the lowest quintile of intake. This study did not lend support for vegetables having a superior effect on psychological health; neither did it support an effect for fruit intake, as the 'cooked-vegetable' and 'fruit' dietary patterns were nonsignificant. Nonetheless, a 'Mediterranean-style' pattern was characterised by regular intake of garlic, peppers, mushrooms, salad greens, pasta, and red wine, so vegetables may have contributed to the effects found. A further study (Sutliffe et al., 2018) (quality score= 60%) found a significant reduction in symptoms of depression after the implementation of a vegetable-based diet for six weeks. However, participants attended weekly group meetings for support and encouragement and this social interaction may have confounded the results.

A longitudinal study (Kingsbury et al., 2016) (quality score=70%) reported that combined greater F&V consumption during a data collection time point in 2002-2008 was associated with lower risk of depression at the next data collection time point two years later in 2004-2011. The significance of the association persisted when adjusted for covariates such as age, gender, socioeconomic status, education, previous time point depression scores and history of depression. However, the inclusion of confounding lifestyle factors of obesity, smoking status and social support in the model reduced the association to be nonsignificant. These findings imply that F&V as a combined construct is associated with symptoms of depression, however, health-related behaviours such as obesity and social support mediate the relationship. Habitually consuming F&V may increase positive health behaviours, which consequently contribute to lower symptoms of depression. As this study explored F&V intake as a combined measure, it is not possible to quantify whether there is support for vegetable consumption having a superior influence on symptoms of depression.

2.3.5 Stress and distress

One study (Lee et al., 2016) (quality score=64%) observed positive changes in stress levels after implementing a twelve-week vegetarian diet. The analysis of self-reported, perceived stress levels at baseline compared to week twelve showed a significant reduction (reduced to 19.57±9.68 points from 22.64±10.73). Concurrently, participants showed improved nutritional-status, demonstrated by significantly improved blood lipid profiles. This study did not quantify daily intake of fruit or vegetables, so effects due to portions of F&V cannot be defined. Furthermore, this study was conducted in Korea and the vegetarian diet implemented involved culturally specific foods, which may limit the transferability to other cultures.

Another study (Kingsbury et al., 2016) (quality score=70%) identified that greater F&V consumption at a baseline time point was associated with lower distress scores two years later. This significant association remained when adjusted for age, gender, history of depression, education, socioeconomic status, chronic disease, binge drinking and obesity. However, when social support, smoking and physical activity were added to the model, the association was no longer significant. The findings indicated that social support was strongly associated with distress, depression, and poor diet, including low F&V consumption. Thus, social support may also moderate the negative impact of low F&V consumption on psychological health. From the evidence presented, the strength and direct causality of vegetable consumption on stress and distress levels remain unclear.

2.3.6 Psychological wellbeing

Evidence from two micro-longitudinal studies provide some support for the hypothesis that vegetable consumption may be more influential in promoting states of positive psychological wellbeing than fruit. One study showed that when current mood was controlled for, vegetable consumption continued to predict psychological wellbeing, but fruit did not for between-person associations (Conner et al., 2015) (quality score=60%). Also, the strength of the associations were stronger for vegetable consumption (r = .174, $p < .001$) compared with fruit consumption (r = .085, $p = .89$). Another study found that both men and women benefitted equally from influences of vegetable consumption on psychological wellbeing, but not for fruit (White et al., 2013) (quality score=55%). Overall, even small increases in F&V consumption were associated with enhanced psychological wellbeing. Vegetable consumption also played a stronger role in changes to psychological wellbeing between and within individuals (Conner et al., 2015). It was found that on days when participants consumed more F&V they reported greater daily eudaimonic wellbeing (social and psychological wellbeing, also referred to as flourishing) and positive affect than on days when they consumed fewer F&V. The wellbeing relationship was within-day as no next-day lagged relationships were found. When daily changes in positive affect were controlled for, vegetable consumption continued to predict increases in daily eudaimonic wellbeing within individuals (fruit also predicted increased wellbeing when holding positive affect constant). Additionally, the consumption of vegetables was associated with lower negative affect, but this was not the case for fruit consumption. In summary, the between-person and within-person associations between vegetables and eudaimonic wellbeing were stronger when compared with fruit.

The other study (White et al., 2013) also applied multi-level modelling analysis to complete repeated daily observations nested within individuals to explore how psychological wellbeing fluctuated as a function of the foods consumed over time. The findings imply that F&V consumption similarly influence psychological wellbeing. On days when individuals consumed 0.15 more portions of vegetables and 0.11 more portions of fruit, they experienced higher positive affect, which included feeling calmer, happier and more energetic. These associations exceeded the adjusted significance threshold of $p < 0.005$. Secondly, lagged analyses revealed that on days when individuals consumed more portions of vegetables or fruit, they reported greater positive affect the next-day (while controlling for current-day fruit/vegetable consumption). However, this lagged finding was not replicated by the aforementioned study (Conner et al., 2015).

The predictor model findings indicated that for every one serving increase in vegetables or fruit (above the participants' habitual intake), positive affect increased by 0.028 or 0.029 points, respectively (White et al., 2013). From these findings, it was predicted that 5.7 additional daily vegetable portions (or 5.5 fruit portions) above habitual daily intake would produce meaningful increases in daily positive affect (reflecting a small Cohen's *d* effect size of 0.2). Taking the participants' baseline daily F&V intakes into account (1.7 portions of fruit; 2.5 portions of vegetables), a daily intake of approximately 7-8 combined F&V portions is required for meaningful change to positive affect (alternatively 8.2 total daily servings of vegetables or, 7.2 total servings of fruit). These findings remained regardless of the BMI (Body Mass Index) of participants, however, while both men and women benefitted equally from vegetable consumption, the association for fruit consumption and positive affect (same-day and next-day) was significantly stronger in men than women. Notably, the reverse causality analysis was not significant; that is, positive affect did not predict vegetable (or fruit) intake. However, from the evidence discussed, it is not possible to conclude that the relationship is causal or direct because these studies did not involve experimental manipulation of F&V intake.

RCT studies have begun to address this gap; findings from the fourteen-day RCT study (quality score=82%) indicated that a relatively small increase in F&V consumption is associated with improvements in psychological wellbeing (Conner et al., 2017). This study showed that after providing young adults with F&V to consume regularly, significant psychological wellbeing gains were observed from consuming +1.2 more F&V portions a day from baseline, or, increasing consumption by 1 portion more than the control group (this study did not delineate separate F&V effects). In regard to effect sizes analysed using variance-explained effect size estimates of the time slopes, the F&V provision condition compared to the control predicted 10.5% of the variance in the growth in flourishing, 8.7% growth in vitality and 18.4% of the growth in flourishing behaviours (motivation, curiosity etc.).

The blood samples taken before and after the RCT intervention validated self-reported increases in F&V intake through increases in biomarkers (vitamin C and plasma carotenoids). However, the increases in vitamin C and carotenoids for the intervention conditions were well-below saturation levels (Brookie et al., 2017), thus psychological wellbeing improvements could have been larger if a higher consumption of F&V had been achieved. The participants were low F&V consumers who typically consumed less than 3 portions of F&V a day. This daily intake is lower than that of the previous study sample, which identified that meaningful change in wellbeing, requires consumption of 7-8 daily portions of F&V (White et al., 2013). However, the RCT findings show that meaningful change to psychological wellbeing can be achieved through approximately one portion increase in F&V intake per day. Participants still fell short of the 5+ a day F&V target (WHO, 2003) with F&V intakes of 3.7 portions per day for the F&V intervention condition compared to 2.8 portions for the control condition. More specifically, average daily portions were 2 for vegetables in the F&V condition versus 1.6 in the control, 1.8 for fruit in the F&V condition versus 1.2 for the control (Brookie et al., 2017). However, the influence of F&V was only evaluated as a combined food category.

Another study (Sutliffe et al., 2018) observed significant improvements in overall quality of life after participants completed a six-week nutrition intervention which included the daily consumption of green vegetables and fruits (a micronutrient-dense, plant-rich diet). However, it is not possible to disentangle the influence of vegetable consumption because participants also reduced their intake of refined/processed foods and animal products. In addition, they received social support through weekly group meetings and were encouraged to take a multivitamin containing B12, iodine, zinc, and Vitamin D.

2.3.7 Lifestyle interventions and psychological health

Two studies involving a community-based lifestyle intervention measured F&V consumption as a combined food category, therefore, it was not possible to delineate vegetable consumption effects from fruit effects. Therefore, the hypothesis that vegetable consumption would have a superior influence on psychological health could not be examined. Nonetheless, both studies provide support for the influence of F&V on psychological wellbeing and imply that the effects are robust for positive psychological health outcomes.

The first study (Johnson et al., 2017) (quality score=73%) examined changes in wellbeing associated with a twelve-week community healthy lifestyle programme, aimed at increasing F&V intake and physical activity. This study showed that increased F&V intake, independent from physical activity, explained some of the change in psychological wellbeing post-intervention and three months later. Changes in F&V intake accounted for the greater change in psychological wellbeing when compared with physical activity; a one-portion increase in F&V intake was associated with an increase of 1.26 positive psychological wellbeing units (measured by the WEMWBS). This was a clinically meaningful improvement even after adjusting for age, gender, physical activity, and walking.

The second study (Blank et al., 2007) (quality score=70%) followed the same population over two years and showed that increasing F&V intake to improve diet quality (mediated by community-level projects) was significantly associated with an improvement in psychological wellbeing. This included less nervousness and reduced feelings of being 'down in the dumps.' The improvements were stronger for positive psychological wellbeing, including happiness and peacefulness. Improved self-rated mental health was associated with an increase in daily consumption of F&V, which only reached statistical significance for those with the greatest improvement in their eating behaviour. Therefore, large margins for change to F&V intake may be required to observe improvements in mental health. Together, this evidence suggests that by increasing F&V consumption over a short time period (3 months) or a longer time period (2 years) that associated improvements in psychological wellbeing will be observed.

2.4 Discussion

This review identified a growing body of prospective research that has investigated the role of vegetable (and fruit) consumption in psychological health. Overall, beneficial effects of habitual vegetable and fruit consumption have been reported, with more consistent findings shown for psychological wellbeing compared to measures of mental health, including symptoms of depression, stress and distress. The results from the studies that focused on psychological wellbeing suggest that consuming more portions of vegetables is associated with higher positive affect, lower negative affect and greater eudaimonic wellbeing. Also, consuming more portions of F&V combined is associated with increases in psychological wellbeing, such as feelings of flourishing and vitality. There was also evidence to suggest a preferential effect of vegetable consumption compared to fruit for psychological wellbeing. This was shown by stronger, more robust associations and consistent benefits of vegetable consumption for both men and women compared to fruit consumption.

Increased vegetable consumption (independent from fruit or, combined with fruit) was found to have enhancing effects on positive states of psychological wellbeing after brief periods of time (13 to 21 days) and longer term (six or twelve weeks to 2 years). This included influences on eudaimonic wellbeing, feelings of flourishing, vitality, motivation, improved positive and negative affect, increased happiness, feeling more energised, calmer and increased overall quality of life. Of the three studies that explored fruit and vegetable intake independently, two explored this in relation to psychological wellbeing and both reported that vegetable consumption increased psychological wellbeing (Conner et al., 2015; White et al., 2013). Suboptimal intakes of nutrients found in vegetables, such as folate, have been suggested as a plausible mechanism to explain the relationship observed between F&V intake and psychological wellbeing (Rooney et al., 2013). Folate consumption influences L-Methylfolate levels in the brain which modulate the synthesis of monoamine neurotransmitters serotonin, norepinephrine and dopamine (Stahl, 2008). These neurotransmitters are robustly associated with mental health and various nutrients can affect these neurotransmitter levels (Rao et al., 2008). Alternatively, F&V consumption may lead to both the expectancy of psychological benefits and consequent improvements in psychological wellbeing. However, one study in this review found that psychological expectancies did not mediate the significant relationship between F&V and psychological wellbeing (Conner et al., 2017).

One question to consider is whether the magnitude of any observed effects are likely to be dependent upon baseline habitual consumption. This review identified evidence that increased F&V intake coincided with improvements to wellbeing in a clinically meaningful, dose-response manner per portion of F&V consumed. However, the levels of intake required for meaningful changes differed between studies, ranging from 3.7-7/8 portions. The low level of intake required for meaningful change was found in low baseline consumers (no more than 3 combined portions of F&V). This suggests that the margin for change is important; lower baseline intakes may yield stronger enhancing effects. This was also identified in the lifestyle change intervention study (Blank et al., 2007), where only participants who showed the greatest improvement to their F&V consumption experienced a significant improvement in self-rated mental health across the two year period. Previous research has observed substantial positive improvements in psychological wellbeing within a two year period when F&V consumption were increased from the lowest levels to the highest levels of intake (Mujcic & Oswald, 2016). The higher level of F&V intake required for meaningful change are similar to Austalia's and Canada's recommended daily intake of 7+ portions (Australian Department of Health, 2009; Government of Canada, 2008). Thus, the findings imply that regularly consuming close to recommended or higher levels of F&V, results in enhanced daily psychological health through subjective psychological wellbeing, and that greater improvements are to be gained when F&V intakes are particularly low.

The evidence to date suggests that a dose-dependent relationship exists between F&V consumption and psychological wellbeing. Previous cross-sectional research has observed this dose-response relationship by assessing how psychological wellbeing differs across different amounts of F&V intake (Blanchflower et al., 2013; Brookie et al., 2018; Hong & Peltzer, 2017; Warner et al., 2017). Higher intakes of F&V are associated with increasingly higher positive psychological health scores. For instance, mean positive affect was found to have a positive linear trend with the number of daily servings of F&V (no association was found for negative affect) (Warner et al., 2017). This association remained statistically significant after the inclusion of demographic, diet and health behaviour variables. A very large increase in F&V (0 portions to 8+ portions) consumption was associated with approximately a 4-point increase (or .57 SD increase) in positive affect (reflecting a medium Cohen's *d* effect size). These findings correspond with the results from this systematic review and it is clear that the margins for increasing consumption are of significance.

The form in which F&V are consumed may influence the effect they have on psychological health. Recently, researchers have begun to compare the associations of raw versus cooked F&V consumption and the findings show that raw F&V consumption predicted reduced depressive symptoms and higher positive mood, life satisfaction, and flourishing (Brookie et al., 2018), However, processed F&V consumption only predicted higher positive mood. In the RCT study identified by this review (Conner et al., 2017), the participants in the F&V intervention consumed the high-quality F&V that they received mostly raw as snacks (approximately 72% of participants). No improvements to psychological wellbeing were observed in the condition that involved text message reminders to increase F&V intakes, despite similar increases to F&V consumption as the provision condition. In this condition, participants reported adding more vegetables to main meals (only approximately 27% of participants consumed raw). This was supported by the blood sample analysis that showed increased plasma carotenoids in the F&V provision condition, but not in the text message reminder condition. In support of this, food preparation has influences on the bioavailability of the micronutrients consumed (Hotz & Gibson, 2007). Hence, when F&V are consumed raw this may influence mental health and psychological wellbeing more strongly. Together these findings may offer some explanation for the mixed results regarding mental health, as studies do not differentiate between raw or processed consumption. The types and processing of the F&V consumed are also important. To date, research suggests that the most influential raw foods related to improved mental health include carrots, bananas, dark leafy greens like spinach and citrus fruits (Brookie et al., 2018), and it is plausible that vegetable consumption may exert stronger influences on wellbeing than fruit through specific bioactive compounds (Brandt et al., 2004).

This review highlights the need for future studies that test the consumption of fruit and vegetables separately, in order to explore possible differential effects on psychological health. Research tends to consider F&V as a combined food group as only three studies evaluated the effects of fruit and vegetables independently. However, the results showed that when measured separately, F&V appear to exert differential effects on psychological health. For instance, vegetables may be more strongly associated with psychological wellbeing. There is the need for future research to consider F&V as separate food categories in order to identify the contributions and most potent targets for psychological health. There was also a wide range of measures used to evaluate mental health across the studies, this reinforces the need for future research to use measures consistent with those previously applied. With further appropriate studies, a meta-analysis would be possible and would help to quantify the different effects across studies.

As this review excluded clinical samples it does not report the influence of vegetable intake as an accessible treatment for the management of psychological issues, however, it should be noted that increasing daily vegetable consumption through a 12-week RCT intervention has been shown to have therapeutic impact in a clinically depressed population by effectively reducing symptoms of depression (Jacka et al., 2017). Further RCT studies that explore reductions in mental health symptoms and wellbeing gains in both clinical and non-clinical populations are called for. These should control for a range of health-related variables, for example physical activity and BMI. It is timely to search for wider, affordable, and modifiable lifestyle behaviours that improve psychological health.

2.5 Conclusion

This review identified that increased F&V consumption has a positive impact on psychological health. The evidence suggests that vegetable consumption may be a more potent target than fruit consumption for enhancing psychological wellbeing and that interventions to increase psychological wellbeing gains should focus on populations where baseline intake is particularly low. However, it must be noted that these conclusions are drawn from limited data. The effect of F&V consumption on mental health is less clear, hence, further work is required, particularly RCT studies that delineate the effect of fruit and vegetables. Overall, based on the limited evidence to date, vegetable consumption is relevant to psychological health and could contribute to lifestyle medicine as an affordable preventative public health care strategy.

Chapter 3 Exploring the Relationship between Dietary Intake, Cognition, and Psychological Health in a UK Adult Sample; A Cross-sectional Study

This chapter was published in The British Journal of Nutrition, only slight adjustments have been made to ensure cohesion within the thesis.

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The initial narrative review (Chapter 1) identified that FVI should be assessed separately, while also considering frequency and quantity of F&V consumption. In addition, the narrative review suggested that the frequency of sweet and savoury snacking may represent habitual dietary intake of macronutrients (sugar and saturated fat), known to have detrimental effects on cognition and psychological health. Furthermore, few studies have considered psychological mechanisms, such as cognitive processes as a mediator in the relationship between dietary intake and psychological health. The previous systematic review (Chapter 2) highlighted the need for more studies that evaluate F&V as separate food categories, in relation to both positive psychological wellbeing and symptoms of poor mental health. Moreover, there is a need for more studies across the lifespan. Therefore, this chapter will address the second aim of the thesis, to investigate the complex relationship within the general adult population, while accounting for key covariates. It will also address the third aim of evaluating whether cognitive processes act as a mediator in this relationship on psychological health, including symptoms of depression, anxiety, stress, and positive psychological wellbeing. Thus, assessment of frequency and quantity of FVI (separately), sweet and savoury snacking, cognitive processes, and a range of aspects of psychological health within healthy adults will follow in this chapter.

3.1 Introduction

The consumption of nutrient-rich (unprocessed) foods, such as fruit and vegetables have been associated with fewer psychological health issues (Lai et al., 2014; Nicolaou et al., 2020) and reduced cognitive impairment (Gomez-Pinilla & Nguyen, 2012). Fruit and vegetable intake (FVI) are also associated with a reduced risk of symptoms of depression, stress, and anxiety (Gibson-Smith et al., 2020; Mikolajczyk et al., 2009), and there is accumulating evidence that FVI is positively related to psychological wellbeing (Mujcic & Oswald, 2016; Ocean et al., 2019). Although studies have begun to explore fruit and vegetable consumption as separate predictors of psychological health (Mihrshahi et al., 2015; Nguyen et al., 2017), few have evaluated the impact of frequency. However, a recent study reported that the frequency with which fruits and vegetables are consumed may be more important than quantity of consumption, suggesting a more nuanced approach to consumption may be required (Ocean et al., 2019).

Conversely, habitual consumption of nutrient-poor (processed) foods, such as sweet and savoury snacks, is associated with increased risk of depression, anxiety, stress (Sangsefidi et al., 2020) and lower psychological wellbeing (Smith & Rogers, 2014). A prospective study reported that trans-unsaturated fatty acid in processed food, such as snack foods (crisps, cookies, cakes etc.), was associated with a higher depression risk at follow up 8-10 years later risk (Sánchez-Villegas et al., 2011), illustrating the potential long-term effects of diet on psychological health. Further, evidence shows that a decrease in frequency of fruit intake, and an increase in frequency of snack food intake, independently coincided with increased perceived stress (Liu et al., 2007). Thus, the frequency of nutrient-poor food consumption may also distinctively contribute to psychological health.

Little is known about the psychological mechanisms by which diet may affect psychological health, the role of cognitive processes (for example, memory and executive function) has been implicated (Monti et al., 2015; Stevenson, 2017). Dietary intake high in calories or low in micronutrients, antioxidants or fibre consumption may reduce optimal brain function, such as neurotransmitter regulation and inflammatory pathways, leading to poorer psychological health (Young et al., 2020). FVI have been shown to enhance cognition (Polidori et al., 2009) whereas saturated fat and sugar intake decrease cognitive performance (Beilharz et al., 2015). Specifically, diet-induced changes to memory and inhibitory control have been consistently observed (Attuquayefio & Stevenson, 2015; Francis & Stevenson, 2011). Frequent cognitive failures (memory errors) have been associated with increased perceived stress and sadness (Boals & Banks, 2012; Payne & Schnapp, 2014). Additionally, reduced inhibitory control is a risk factor for depression (Gotlib & Joormann, 2010; Kaiser et al., 2003). Even subtle cognitive deficits are significantly associated with reduced psychological wellbeing (Gates et al., 2014), therefore, dietary intake may impact cognition and in turn psychological health.

Research has begun to identify relationships between the consumption of nutrient-rich or nutrient-poor foods and psychological health. However, it is important to evaluate the independent associations of the frequency and quantity of FVI, frequent snacking on energy-dense foods, and the role of cognitive processes as a potential mediator. This study aimed to assess the direct and indirect relationship between dietary intake and depression, anxiety, stress, and wellbeing. It was predicted that greater nutrient-rich FVI (as separate frequency and portions variables) would be associated with increased psychological health, whereas greater nutrient-poor sweet and savoury snacking would be associated with decreased psychological health. It was also predicted that cognitive failures and inhibitory control would mediate the relationship between dietary intake and psychological health, whereby FVI would negatively predict cognitive failures and positively predict inhibitory control scores and thus increase psychological health, whereas sweet and savoury snacking would positively predict cognitive failures, negatively predict inhibitory control, and thus reduce psychological health.

3.2 Method

Design

This is a cross-sectional design; participants completed measures of demographic, health, and lifestyle behaviour, eating behaviour, dietary intake, mental health, psychological wellbeing, and cognition, at one point in time via an online platform (www.gorilla.sc).

Participants

Participants were recruited using the recruitment platform Toluna to gain access to a nationally representative sample of the UK. Toluna is an online community website which invites members to complete paid surveys. Participants were compensated with 3,000 Toluna points (redeemable towards retail vouchers) after completing the survey. A total of 977 participants provided informed consent to take part, with 442 participants completing this online cross-sectional study. Of these, 14 were excluded from analyses due to having a go Reaction Time (RT) percentage accuracy lower than 60% on the inhibitory control task (see below for more information on this task), thus the total n for analysis = 428 (53% were female). Participants completed an initial screener questionnaire to ensure they met the inclusion criteria which included being aged 18 - 60 years of age, not colour blind (due to the demands of the inhibitory control task) and having English as their first language. Participants who rated their general health as poor over the last 12 months, who currently have or had diabetes, or an eating disorder and/or medically diagnosed food allergy, high blood pressure, a heart attack, or were experiencing medical illness were not eligible to participate (509 participants did not meet the screening criteria and 26 eligible participants left the survey incomplete). The study was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample Size

Using G*Power, with alpha set at 0.05, modelling a small effect size, and power at 80%, the minimum required sample size was 395 participants (Faul et al., 2009). However, to account for participants who might not complete the study in full, or whose data may need to be excluded from analysis (e.g., due to the threshold for the inhibitory control task), we aimed to enrol a minimum of 450 participants onto the study.

Procedure

The Gorilla Experiment platform was used to create and host the cross-sectional study. The survey and cognitive task were completed by participants online using a computer; phones or tablets were not permitted because the cognitive task required the use of a keyboard. Participants who expressed a willingness to take part provided informed consent and were screened for the inclusion criteria. Eligible participants then progressed through the series of questionnaires measuring demographic information, dietary intake, lifestyle behaviours, psychological health, cognition, mood, and appetite, followed by the stop-signal task, and were finally debriefed.

Measures

Lifestyle behaviours, health, and demographic information: During an initial screener questionnaire, general health was assessed using a single item with a five-point Likert scale (ranging from poor to excellent) asking participants to rate: “Over the last 12 months, would you say that on the whole, your health has been…”. Participants were also asked if they previously or currently had diabetes, an eating disorder, a medically diagnosed food allergy, high blood pressure, a heart attack, or any other medical illness to recruit healthy adults. Sex, age, and ethnic group were also collected to characterise the sample.

Within the Short-form Food Frequency Questionnaire (SF-FFQ; see below), there were items that assessed average weekly alcohol intake, exercise, and smoking status and these were assessed as potential lifestyle variable covariates. Exercise (total minutes last week) was used in the present study as a control variable for the mediation models, given the evidence that physical activity has beneficial effects on cognitive function (Hötting & Röder, 2013; Molteni et al., 2004) and psychological health (Schuch et al., 2016).

Additionally, the 21-item, four-point Likert Three Factor Eating Questionnaire (TFEQ) (Cappelleri et al., 2009) was used to collect data on eating style to calculate an uncontrolled eating, cognitive restraint, and emotional eating average score for the sample. Participants were also asked if they were vegetarian or vegan. To calculate Body Mass Index (BMI), participants were asked to report their height and weight in either metric or imperial units.

Dietary intake: The Short-form Food Frequency Questionnaire (SF-FFQ) was used to examine the consumption of foods in a "typical" week, over the past month by measuring the frequency of food group consumption using an 8-point Likert scale (rarely or never to 5+ a day) (Cleghorn & Cade, 2016; University of Leeds nutritools.org). The food groups investigated in this study were: (1) fruit consumption, which included fresh or tinned; (2) vegetable consumption, which included fresh, tinned, or frozen, but not potatoes; (3) sweet snacking, which included biscuits, cakes, chocolate, and sweets, and (4) savoury snacking, which included crisps or savoury snacks. In addition to these measures of frequency of consumption, the SF-FFQ included two additional single items to collect data on average fruit and vegetable consumption per day in portions. One portion was quantified as approximately 80g in weight and examples were provided for each item, such as "a handful of grapes". The measure did not include similar items for sweet or savoury snacking; however, this questionnaire is valid and reliable for assessing diet in the UK population, hence, was used here (Cleghorn et al., 2016). Validation of this measure has shown that participant SF-FFQ responses for single food items are independently predictive of a participant's diet quality score (as measured by a 217 item FFQ used in the UK Women's Cohort Study) (Cleghorn et al., 2016). This highlights that individual food groups are significant for dietary assessment.

Psychological health: Symptoms of depression and anxiety during the past week were measured using the 14-item, four-point Likert, Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). The HADS was designed as a screening tool to identify possible and probable cases of anxiety and depression in outpatients (Bjelland et al., 2002). Stress was measured using the 10-item, five-point Likert, Perceived Stress Scale (PSS) (Cohen et al., 1983). The items examine levels of stress experienced over the last month. Visual analogue scales (VAS) were used to assess current mood and appetite ratings (Crichton, 2001). Participants indicated on a 100mm horizontal line the point that represented their current experience of the following (where 0 = not at all, and 100 = very much): sad, happy, anxious, alert, drowsy, withdrawn, and hungry. Positive psychological wellbeing was measured using the 14-item, five-point Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS), which is validated for use in the general adult population (Tennant et al., 2007). The WEMWBS evaluates subjective wellbeing and psychological functioning over the last two weeks (Stewart-Brown & Janmohamed, 2008). The WEMWBS does not a have a ‘cut off’ level to indicate those who have ‘good’ and those who have ‘poor’ psychological wellbeing, but the minimum score is 14 and the maximum is 70 (Stewart-Brown & Janmohamed., 2008).

Cognitive processes: The Cognitive Failures 25-item self-report questionnaire (CFQ) measured attentional, **memory, perceptual and action-related mental lapses in everyday tasks** over the past six months (Broadbent et al., 1982). One item was adapted: “Do you leave important letters unanswered for days?” whereby “letters” was changed to “emails.” The CFQ was used to assess the frequency of global cognitive dysfunction. Scores can range from 0 to 100, whereby higher scores indicate more subjectively experienced cognitive failures. The CFQ has high internal validity (alpha=0.91) and test-retest reliability (Wallace et al., 2002). The CFQ has been correlated with the Everyday Memory Questionnaire (r = -.64) which assesses memory errors (Martin, 1983). Furthermore, CFQ items load on three different factors, forgetfulness, distractibility, and false triggering (Rast et al., 2009).

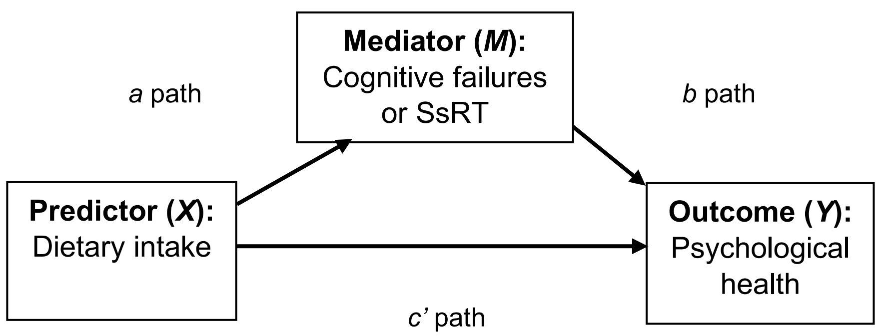
The stop-signal task (SST) is a behavioural measure of **inhibitory control** which was used to provide a Stop-signal Reaction Time (SsRT); this measure represents a participant’s efficiency to inhibit an already initiated response (Logan, 1994). Higher SsRTs indicate poorer inhibitory control and the SST has been shown to be one of the most sensitive and reliable measures of executive control (Padilla et al., 2013). This task-based measure of inhibitory control may provide insight into the specific neural and behavioural impairments of cognition associated with both eating and the development or maintenance of poor psychological health (Bartholdy et al., 2016; Coumans et al., 2018; Marazziti et al., 2010). An online SST was created using The Gorilla Experiment Builder (Anwyl-Irvine et al., 2019) based on the stop-signal paradigm (Verbruggen & Logan, 2009). The SST included a practice task with 16 trials whereby participants were required to reach an accuracy threshold of 50% to proceed to the main task. The main task comprised 6 blocks of 16 trials (96 trials in total, 75% go trials, 25% stop-signal trials). Each trial started with the presentation of a fixation cross which was replaced with an arrow pointing in the left or right direction inside a white circle (the inter-stimulus interval was 500 milliseconds (msec)). Participants indicated the direction of the arrow using the keyboard responses: ‘b’ for left and ‘n’ for right (the maximum response time was 1,500 msec before the trial moved along). On stop-signal trials, the white circle surrounding the directional arrow changed to red which occurred after a variable delay ranging from 250-400 msec, with 50 msec incremental increases in difficulty, and participants were required to withhold a response on these trials. The function to staircase difficulty (in response to participants’ inhibition performance as per

Verbruggen et al., 2019) and establish the interval for which participants inhibited their responses with an accuracy of 50% (in order to individually challenge participants) was not available using the Gorilla Experiment platform.

Statistical analyses

Descriptive data were calculated as means with standard deviation, or as frequencies (%) for categorical variables. For the frequency of dietary intake, the median and range is presented. For processing the cognitive task raw data, a published protocol was applied to estimate SsRT and the average SSD (stop-signal delay) (Congdon et al., 2012). Simple linear and stepwise multiple regression analyses were conducted to explore the relationship between dietary intake and psychological health (before and after including the covariates: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake). The selection of these covariates was informed by previous literature (Gibson-Smith et al., 2020; Mihrshahi et al., 2015; Ocean et al., 2019). The stepwise analysis put predictors into competition with each other by applying the ≤ 0.050 to enter and ≥ 0.1 to remove criteria. Separate models were conducted for dietary intake (food frequency) for fruit, vegetable, sweet and savoury snack consumption as predictors. Fruit and vegetable intake (separately) quantified as portions consumed were also explored as separate predictors. The outcome variables included four different measures of psychological health: symptoms of depression, anxiety, stress, and positive psychological wellbeing scores. Multiple mediation analyses were performed using PROCESS, version 3.5 (Hayes, 2017) in SPSS version 26 using bootstrapping over 5,000 samples. The mediators included in the models were either cognitive failures score or SsRT (the index of inhibitory control). All mediation models included covariates that were consistently, significantly associated with the psychological health outcomes at the $p < 0.01$ level, which were general health rating and exercise (see Figure 3.1 for the mediation paths).

Figure 3.1 Mediation paths. All models controlled for general health rating and exercise.



3.3 Results

3.3.1 Descriptive Results

Participant characteristics: Descriptive statistics regarding demographic and lifestyle behaviour information for the healthy adult sample are presented in Table 3.1. On average the participants were middle aged, 52.8% of the sample were female and 89.7% identified as white for ethnicity. The mean BMI for the sample was 26.0 (*SD* = 5.6) and 53.5% were healthy weight. On average, participants exercised for 2.5 hours per week and most of the sample rated their general health as good to excellent (86%). Further, 63.1% were not smokers and 79.2% consume less than 14 units of alcohol per week or rarely/never drink. Finally, education and household income information were provided by some of the sample (n= 207), and of these, the majority achieved University level education (66% of the sample) and their household income was between £20,000 and £49,999 (73% of the sample).

Table 3.1 Sample demographic information and lifestyle behaviour (n = 428).

Characteristics	*N/Mean(SD)*	Percentage/range
Mean age in years (SD)	39.7 (13.0)	18-60
Sex		
Female	226	52.8
Male	200	46.7
Other	2	0.5
Ethnicity/Race		
White	384	89.7
Asian	8	1.9
Black	13	3.0
Chinese	7	1.6
Mixed	10	2.3
Other	5	1.3
Prefer not to say	1	.2
Mean body mass index (BMI)	26.0 (5.6)	14-50
Underweight	12	2.8
Healthy weight	229	53.5
Overweight	103	24.1
Obese	78	18.2
Prefer not to say	6	1.4
Mean exercise (total minutes) (SD)	152.9 (151.1)	0-840
General health rating		
Excellent	62	14.5
Very good	167	39.0
Good	140	32.7
Fair	59	13.8
Smoking		
Never smoked > 100 cigarettes	270	63.1
Current smoker	77	18.0
Ex-smoker	81	18.9
Alcohol intake (units per week)		
Rarely or never drink	170	39.7

< 14 units	169	39.5
14-21 units	56	13.1
> 21 units	33	7.7
Eating style (TFEQ)		
Uncontrolled eating score	2.4 (0.6)	1-3.8
Cognitive restraint score	2.6 (0.5)	1-3.8
Emotional eating score	2.6 (0.9)	1-4.0

Note: *SD* = standard deviation; BMI n = 422 because 6 participants selected "prefer not to say" National Health Service (NHS) classifications for BMI ranges were used; Underweight < 18.5, Healthy weight 18.5-24.9, Overweight 25-29.9, Obese ≥ 30; Eating style (TFEQ) average scores for each dimension (uncontrolled eating, cognitive restraint, and emotional eating) indicate a non-disordered sample.

Dietary intake: The frequency of dietary intake showed that on average, both sweet and savoury snacks were each consumed 2-3 times a week, while both fruit and vegetables were each consumed 4-6 times a week. The range of responses was from rarely or never to 5+ times a day; overall participants were not consuming fruit and vegetables frequently enough to reach recommended intake. On average, participants consumed 1.9 portions of fruit per day (SD = 1.5) and 2.3 portions of vegetables per day (SD = 1.5), thus combined consumption was short of the recommended daily intake. Fruit and vegetable intake ranged from 0 to 10 portions per day and ten percent of the sample identified as vegetarian or vegan.

Cognition: For the CFQ, the average frequency, variance and range of cognitive failures scores were as follows: mean = 34.8, SD = 16.2, range = 0-97. For the SST, fifty-three participants (12% of the sample) were required to complete the practice trials a second time to meet the accuracy threshold of 50% and proceed to the test phase. However, descriptive results from the test phase showed that on average, the percentage accuracy on stop-signal and go trials was high (stop-signal trials, mean = 81%, SD = 23%; go trials, mean = 93%, SD = 9%). Finally, calculation of the SsRT revealed a mean of 151.4msec (SD = 276.1msec). When comparing these SST variables with other studies, the go trial results are similar to other healthy adult populations, however, the stop-signal trial results suggest that the SST may not have been as challenging as laboratory-based tasks (Padilla et al., 2013).

Psychological health: Descriptive statistics for mood ratings and psychological health are presented in Table 3.2. VAS scores show that on average participants were happy, alert, low in sadness and anxiousness and not highly drowsy or withdrawn. Overall, mean depression and anxiety scores were considered non-clinical and stress levels were moderate (Bjelland et al., 2002; Andreou et al., 2011). The mean psychological wellbeing score (46.0) is similar to other UK general population groups (Bartram et al., 2011). The WEMWBS does not a have a 'cut off' level to indicate those who have 'good' and those who have 'poor' psychological wellbeing, but the minimum score is 14 and the maximum is 70 (Stewart-Brown & Janmohamed, 2008).

Table 3.2 Descriptive results for mood ratings and psychological health (n = 428).

Outcome	Mean (SD)	Range
Sad (VAS)	26.2 (25.2)	0-100
Happy (VAS)	61.7 (23.2)	0-100
Anxious (VAS)	28.0 (26.6)	0-100
Alert (VAS)	68.3 (22.1)	0-100
Drowsy (VAS)	36.0 (26.8)	0-100
Withdrawn (VAS)	33.2 (29.1)	0-100
Hungry (VAS)	31.2 (28.2)	0-100
Depression score (HADS)	6.8 (3.6)	0-18
Anxiety score (HADS)	7.3 (4.5)	0-21
Stress score (PSS)	16.9 (7.8)	0-40
Psychological wellbeing (WEMWBS)	46.0 (10.3)	14-70

3.3.2 Regression Results

Simple linear regression: Prior to the inclusion of covariates, simple linear regression was conducted to analyse sweet and savoury snacking, fruit, and vegetables (frequency or portions) consumed as predictors of either depression, anxiety, stress, or wellbeing scores. Frequency of fruit consumption and portions of vegetables consumed negatively predicted depression scores (β = -.140, p < 0.05, R^2 = .020; β = -.108, p < 0.05, R^2 = .012, respectively). The results also revealed that frequency and portions of fruit consumption positively predicted psychological wellbeing scores (β = .192, p < 0.001, R^2 = .037; β = .120, p < 0.05, R^2 = .014, respectively). Furthermore, sweet, and savoury snacking positively predicted anxiety scores (β = .098, p < 0.05, R^2 = .010; β = .186, p < 0.001, R^2 = .035, respectively), while only savoury snacking positively predicted stress (β = .136, p < 0.05, R^2 = .019). Frequency of vegetable consumption did not predict psychological health (all ps > 0.05).

Stepwise regression: To evaluate the relative contribution of dietary intake (sweet and savoury snacking, frequency and portions of fruit and vegetable consumption) as predictors of psychological health, compared to other known predictors, the following covariates were included in a stepwise multiple regression: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake. The significant stepwise regression models' beta values, p values, change in R^2, and the adjusted R^2 for each step of the analysis are presented in Table 3.3. Firstly, for depression scores, the significant stepwise model selected exercise for entry first (β = -.112, p = 0.021, R^2 = .027), then age was added (β = -.180, p < 0.001, R^2 = .047), followed by general health rating (β = -.128, p = 0.008, R^2 = .068), smoking status (β = -.112, p = 0.019, R^2 = .084), and finally fruit frequency (β = -.109, p = 0.025, R^2 = .095). Overall, the model predicted 8.4% of the variance in depression scores (F (5, 416) = 8.735, p < 0.001, adjusted R^2 total = .084). Secondly, for wellbeing scores, the significant stepwise model selected general health rating for entry first (β = .255,

$p < 0.001$, $R^2 = .064$), then fruit frequency ($\beta = .187$, $p < 0.001$, $R^2 = .096$), and finally age ($\beta = .140$, $p = 0.003$, $R^2 = .116$). Overall, the model predicted 10.9% of the variance in wellbeing scores (F (3, 418) = 18.199, $p < 0.001$, adjusted R^2 total = .109). Finally, for anxiety scores, the significant stepwise model selected age for entry first ($\beta = -.311$, $p < 0.001$, $R^2 = .105$), then general health rating was added ($\beta = -.136$, $p = 0.003$, $R^2 = .130$), followed by smoking status ($\beta = -.139$, $p = 0.002$, $R^2 = .148$), savoury snacking ($\beta = .127$, $p = 0.005$, $R^2 = .163$), and finally sex ($\beta = .101$, $p = 0.029$, $R^2 = .172$). Overall, the model predicted 16.2% of the variance in anxiety scores (F (5, 416) = 17.296, $p < 0.001$, adjusted R^2 total = .162).

Table 3.3 The significant stepwise regression models for the relationship between fruit frequency, savoury snacking, and psychological health, including covariates (n = 428).

Depression	***Exercise***	***Age***	***General health rating***	***Smoking status***	***Fruit frequency***
Unstandardised beta	-.003	-.050	-.507	-.519	-.188
Standardised beta	-.112	-.180	-.128	-.112	-.109
p value	.021	<.001	.008	.019	.025
	Step 1	*Step 2*	*Step 3*	*Step 4*	*Step 5*
R, R²	.164, .027	.216, .047	.262, .068	.290, .084	.308, .095
R² change	.027	.020	.022	.016	.011
Adjusted R²	.025	.042	.062	.075	.084
Wellbeing	***General health rating***	***Fruit frequency***	***Age***		
Unstandardised beta	2.888	.916	.110		
Standardised beta	.255	.187	.140		
p value	<.001	<.001	.003		
	Step 1	*Step 2*	*Step 3*		
R, R²	.254, .064	.310, .096	.340, .116		
R² change	.064	.032	.019		
Adjusted R²	.062	.092	.109		
Anxiety	***Age***	***General health rating***	***Smoking status***	***Savoury snacking***	***Sex***
Unstandardised beta	-.108	-.677	-.805	.362	.898
Standardised beta	-.311	-.136	-.139	.127	.101
p value	<.001	.003	.002	.005	.029
	Step 1	*Step 2*	*Step 3*	*Step 4*	*Step 5*
R, R²	.324, .105	.361, .130	.385, .148	.403, .163	.415, .172
R² change	.105	.025	.018	.014	.010
Adjusted R²	.103	.126	.142	.155	.162

Note: Depression: Step 1 exercise; Step 2 age was added; Step 3 general health rating added; Step 4 smoking status added; Step 5 fruit frequency added. Wellbeing: Step 1 general health rating; Step 2 fruit frequency was added; Step 3 age added. Anxiety: Step 1 age; Step 2 general health rating was added; Step 3 smoking status added; Step 4 savoury snacking added; Step 5 sex added.

Focussing on dietary intake within these models, fruit frequency was a significant negative predictor of depression scores (β = -.109, p = 0.025) and independently contributes 1.1% to the variance explained (R^2 change value at step 5 = .011), with an adjusted, standardised beta estimate of -.099 (after adjusting for exercise, age, general health rating and smoking status retained in the significant stepwise model). Fruit frequency was also a significant positive predictor of positive psychological wellbeing scores (β = .187, p < 0.001) and independently contributes 3.2% to the variance explained (R^2 change value at step 2 = .032), with an adjusted, standardised beta estimate of .180 (after adjusting for general health rating and age retained in the significant stepwise model). The unstandardised beta values (presented in Table 3) show that for every 1 unit increase in the frequency of fruit consumption (for example, from 4-6 times a week to 1-2 times a day), depression scores decrease by .188, while positive wellbeing scores increase by .916. Furthermore, savoury snacking was a significant positive predictor of anxiety scores (β = .127, p = 0.005) and independently contributes 1.4% to the variance explained (R^2 change value at step 4 = .014), with an adjusted, standardised beta estimate of .131 (after adjusting for age, general health rating, smoking status and sex retained in the significant stepwise model). Thus, for every 1 unit increase in the frequency of savoury snacking (for example, from 2-3 times a week to 4-6 times a week), anxiety scores increase by .362. After including the covariates in the stepwise regression, all other models for snacking, vegetable and fruit portions were no longer significant predictors of psychological health (all *p*s > 0.05).

3.3.3 Mediation Results

Mediation analyses were applied to follow-up on the significant regression analyses presented above. Cognitive failures mediated the relationship between savoury snacking and psychological health whilst controlling for general health rating and exercise. Specifically, savoury snacking significantly positively predicted cognitive failures (*a* pathway; B = 1.93, p < 0.001). As cognitive failures increased, so did depression (*b* pathway; B = 0.10, p < 0.001), stress (*b* pathway; B = .23, p < 0.001) and anxiety scores (*b* pathway; B = .16, p < 0.001), whereas wellbeing scores decreased (*b* pathway; B = -.23, p < 0.001). Savoury snacking was not a direct significant predictor of psychological health when cognitive failures were held constant (for all *c'* pathways, p > 0.05), however, savoury snacking was a significant predictor of stress (p < 0.05) and anxiety (p < 0.001) when the indirect and direct pathways were combined (*c* total pathway). Further, examining the indirect effect (*ab* pathway) indicated that mediation had occurred for: depression (*ab* = .188, 95% CI [.082, .309]); anxiety (*ab* = .295, 95% CI [.134, .472]); stress (*ab* = .436, 95% CI [.196, .691]), and; wellbeing (*ab* = -.433, 95% CI [-.721, -.188]). No further significant mediation was identified, either for the other predictors (dietary predictors) or for the other theoretical mediator (inhibitory control). The models did reveal that higher SsRT (indicative of poorer efficiency inhibiting a response) was associated with a significant increase in depression ($p < 0.001$) and stress ($p < 0.05$) scores.

3.4 Discussion

This study assessed the direct relationship between dietary intake and psychological health, while extending existing literature by evaluating both frequency and portions of fruit and vegetable consumption, separately. It also explored a novel mediation model to evaluate the indirect relationship between dietary intake and psychological health via cognitive processes. Results showed that after including a range of covariates, fruit frequency negatively predicted depression scores and positively predicted psychological wellbeing scores. By contrast, savoury snacking positively predicted anxiety scores. Although, cognitive failures did not mediate the relationship between either FVI or sweet snacking and psychological health, mediation was observed for savoury snacking, whereby more frequent consumption of savoury snacks was associated with greater cognitive failures, and in turn, reduced psychological health, including increased symptoms of depression, stress and anxiety, and lower positive psychological wellbeing. Inhibitory control did not mediate any of the relationships between dietary intake and psychological health. The results emphasise that frequency of fruit consumption and savoury snacking could be potential targets for improving psychological health at the individual level, which in turn could have larger gains (e.g., health, social, economic) at a population level.

The positive relationship between FVI and psychological health has been consistently reported in the literature (Blanchflower et al., 2013; Saghafian et al., 2018), however, few studies have analysed FVI separately (Dharmayani et al., 2021; Tuck et al., 2019) and even fewer have compared frequency and portions (quantity) (Ocean et al., 2019). The current results provide further nuance by revealing that the frequency with which fruit is consumed, but not the portions consumed during a typical week, negatively predicted depression and positively predicted psychological wellbeing, after including covariates. This suggests that how often we consume fruit may be more important than the total amount we consume. Indeed, poor mental health has been associated with less frequent intake of fresh fruits among women and men (Sarlio-Lähteenkorva et al., 2004). Additionally, frequent consumption of fruit snacks over a two week period has been shown to improve positive psychological wellbeing (Conner et al., 2017). Hence, the present findings support the notion that frequently consuming nutrient-rich fruits may be more important than the quantity of consumption for psychological health (Ocean et al., 2019), however, experimental studies, varying frequency and portions of fruit consumed, are required to test this directly.

Although fruit frequency predicted psychological health, it is notable that neither the frequency of consumption, or the portions of vegetables consumed, were significant predictors when including age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake as covariates. This highlights both the importance of covariate analysis, but also, the significance of assessing fruit and vegetable intake individually. Furthermore, FVI are often consumed in different environmental contexts as vegetables are typically found to be consumed with family members at home during meal times, while fruits are typically consumed as snacks outside the home and throughout the day (Chawner et al., 2020). Although the null association for vegetables and psychological health was not predicted, it is not necessarily surprising. Recent research suggests that the relationship between FVI and psychological health is stronger for raw fruit and vegetables compared to cooked or canned (Brookie et al., 2018), and that frequent intake of fruit may involve greater consumption in raw form (for instance, snacking on whole fruits), which may maximise the absorption of nutrients with antioxidant properties, thus having a more potent influence on psychological health (Rooney et al., 2013).

Further, precise quantities (daily portions) of vegetable intake may be required to observe an influence on psychological health. Previous research demonstrated that an association between vegetable portions consumed and reduced symptoms of depression, only occurred at higher levels of intake every day (⩾5.0 portions/day) (Mihrshahi et al., 2015). Thus, the current sample's low average vegetable consumption (2.3 portions a day), offers an alternative explanation of the null association, here. Further, recent findings suggest that certain types of fruits and vegetables may be more effective in reducing symptoms of depression. For instance, intakes of tomatoes, dark-green vegetables, berries and fruits were more strongly negatively related to symptoms of depression, than other vegetables and dried fruits (Sun et al., 2021). Therefore, higher quantities of certain categories of vegetables may need to be consumed in order to observe benefits to psychological health.

In contrast to the patterns described above, savoury snacking positively predicted anxiety scores (but neither FVI nor sweet snacking predicted anxiety scores). Previous research has explored snacking more broadly by combining sweet and savoury intake (Sangsefidi, et al., 2020), thus the current results shed light on a specific link between savoury snacking and anxiety, after including covariates. These results support previous work demonstrating that poorer dietary intake including higher salty snack and fast-food consumption is associated with greater anxiety (Zahedi et al., 2014). Of course, it is necessary to point out that the direction of this relationship is uncertain, as a strategy to cope with anxiety often involves increased consumption of nutrient-poor foods (Adan et al., 2019). However, it may be the case that savoury snacking is more problematic for psychological health than sweet snacking because the current results suggest a robust direct and indirect relationship exists. Hence, similar to fruit and vegetables, assessing the individual contributions of sweet and savoury snacking to our psychological health, appears to be warranted.

Following on from this finding, we also revealed that more frequent savoury snacking was associated with an increase in cognitive failures, which were in turn associated with increased symptoms of depression, stress, and anxiety, but also a decrease in wellbeing. This mediation supports previous findings that snacking on nutrient-poor processed foods, such as crisps, is associated with increased cognitive and psychological problems (Sangsefidi et al., 2020; Smith & Rogers, 2014) and builds on findings that reported a positive relationship between frequent unhealthy snacking and higher cognitive failures and stress (Chaplin, & Smith, 2011). Research shows that subjective memory concern during daily activities is a significant predictor of psychological wellbeing, anxiety, and symptoms of depression (Gates et al., 2014). It is possible that savoury snacking may have a negative effect on psychological health, via a decrease in cognition related to general memory errors due to saturated fat content (Francis & Stevenson, 2011; Gibson et al., 2013). There is an abundance of evidence from animal models showing that high saturated fat diets specifically reduce memory function (Wu et al., 2004) which speaks to the causal link between diet and cognition. Hence, the memory errors reflected in the cognitive failures measure, may be the cognitive mechanisms by which processed food intake indirectly reduces psychological health. Indeed, that inhibitory control did not mediate this effect may suggest that specific cognitive processes are involved, relating to memory.

Strengths of this study included that it was a large national sample of the UK population, including a wide participant age range with gender balance. Also, instead of a general measure of psychological health, multiple aspects were measured as discrete outcomes (depression, anxiety, stress, positive psychological wellbeing), and similarly, mutiple aspects of dietary intake were assessed (fruit and vegetables separately; frequency and portions, etc.) providing greater precision. Nevertheless, there are limitations to be considered. This study provides a credible model by which we can infer causal relationships, but due to the cross-sectional design, further prospective and experimental work is required to test causality and temporality robustly. Furthermore, the food frequency measure did not identify the number of snacks consumed, therefore the portions of sweet and savoury snacks consumed was not evaluated. Finally, the testing environment could not be precisely controlled, as this was an online study, thus the results must be considered with that caveat in mind. However, the results suggest that psychological health can be directly and indirectly influenced by specific nutrient-rich (fruit) and nutrient-poor foods (savoury snacks) which contributes to an evidence base for developing effective preventive strategies in public health.

3.5 Conclusion

This study identified that frequent fruit consumption has a direct positive relationship, whereas savoury snacking has a direct negative relationship, with elements of our psychological health. This study also revealed that cognitive processes (cognitive failures) may be one of the mechanisms by which our dietary intake affects our psychological health, but this appears to be limited to savoury snack foods. These results provide new insights on the independent associations between certain types of food and psychological health, and the psychological mechanisms that may mediate these. Future work is needed that replicates this cross-sectional study, and to explore the relationship between dietary intake and psychological health over time.

Chapter 4 Exploring the Relationship between Dietary Intake, Diet Quality, Cognition, and Psychological Health in a University Student Sample; A Cross-sectional Study & A Prospective Study

The previous chapter involved study 1 of the thesis which explored the relationship between nutrient-rich FVI and nutrient-poor sweet and savoury snacks and psychological health, with the novel mediator of cognitive processes. The narrative review (Chapter 1) identified that FVI should be assessed independently and alongside overall diet quality. The following study (study 2) replicates the previous cross-sectional study (study 1), then extends this by computing overall diet quality scores alongside dietary intake variables. The relationships are also investigated over four months in a prospective study (study 3). This expands the prospective evidence base, which was identified as insufficient within the previous systematic review (Chapter 2). Again, studies 2 and 3 explored cognition as a potential mediator of the relationship between FVI and psychological health. The same dietary, cognition (excluding inhibitory control) and psychological health measures were used as the previous cross-sectional study (Chapter 3) to assess replicability. Thus, this chapter addresses the second and third aims of the thesis, which were to investigate the complex relationship between nutrient-rich FVI separately, nutrient-poor sweet and savoury snacks (and overall diet quality), and psychological health, both cross-sectionally, and over time also. The studies also evaluate whether cognitive processes act as a mediator in this relationship on psychological health, including symptoms of depression, anxiety, stress, and positive psychological wellbeing.

4.1 Introduction

Poor psychological health is on the rise in young adults, and this is the largest threat to health, productivity and wellbeing in this age group, accounting for 45% of the overall burden of disease (Gore et al., 2011; McGorry et al., 2022). The transition to university is a period of increased risk of onset of mental health problems, including depression and anxiety (Beiter et al., 2015; Kessler et al., 2007). Approximately 30% of young adults (aged 18 to 25 years of age) report experience of a mental illness, which is higher than all other age groups (Merlo & Vela, 2022). At the same time, young adults consistently report low diet quality, including inadequate FVI (Collins et al., 2022). NHS England (2020) data shows that only 13% of 15-29-year-olds consume the recommended 5-a-day (Ocean et al., 2019). Moreover, snacking accounts for a substantial part of young adults' total energy intake and is one of the most important descriptors of eating habits in this age group (Howarth et al., 2007; Piernas & Popkin, 2010). Thirty-two percent of young adults report habitual snacking on nutrient-poor foods, such as chocolate bars, sweets, crisps, and other savoury snacks, and concerningly this occurs as frequently as six or more times a day (Poobalan et al., 2014). Therefore, young adulthood is an important period for establishing dietary behaviour which will carry across the lifespan, and there is an opportunity to protect psychological health through dietary intake, if a robust relationship is established (Rossa-Roccor et al., 2021).

The link between dietary intake and psychological health has been less studied in young adults, and prospective studies are especially lacking (Dharmayani et al., 2021; Saha et al., 2023). Very few studies have focussed on university students to examine the role of dietary intake and quality in promoting psychological health over time (Kundu et al., 2022). Research reviews identify a significant, negative relationship between F&V consumption (combined or independent) and depression scores (Glabska et al., 2020; Saghafian et al., 2018). Low FVI has been associated with greater experiences of distress and perceived stress in university students (El Ansari et al., 2015; Liu et al., 2007; Mikolajczyk et al., 2009). When FVI increases over time in young adults, corresponding improvements in overall mood and psychological wellbeing are observed (Carr et al., 2013; Conner et al., 2015). In a cross-sectional web-based study of medical students, greater FVI has been associated with higher happiness scores (Lesani et al., 2016), and prospectively with greater psychological wellbeing and increased positive affect the next day (Conner et al., 2017; White et al., 2013). By contrast, a deleterious relationship between nutrient-poor foods and stress, anxiety, and symptoms of depression has been observed in young adult samples, but of course this may be bi-directional (ElBarazi & Tikamdas, 2023; Zahedi et al., 2014), as mental health issues could impact dietary choices too (Lyzwinski et al., 2018). Establishing whether a consistent relationship between dietary intake and/or overall diet quality with psychological health exists in the young adult, university population, will inform the focus of preventative psychological dietary interventions. Early adulthood has been highlighted as an essential stage of life for developing food habits and literacy, such as understanding nutritional recommendations and the connections between food and health (Malan et al., 2020). There may be an opportunity to simultaneously enhance both dietary intake and psychological health, by only targeting diet.

Adherence to a high-quality diet has been associated with better cognitive and mental health, and the opposite for following a low-quality diet (e.g., a Western diet), which includes low intake of F&V and high intake of processed, junk foods (Jacka et al., 2015; Solomou et al., 2023). Moreover, a recent systematic review suggests that habitual saturated fat and sugar intake, often due to high intakes of processed snacks, may modestly impair adult memory function (diet-induced hippocampal impairment) (Taylor et al., 2021). Consumption of nutrient-poor snacks have been associated with increased cognitive and psychological problems (Sangsefidi et al., 2020; Smith & Rogers, 2014). However, few studies have investigated the links between foods high in these macronutrients (e.g., sweet, and savoury snacks) and cognition in young adults who do not show cognitive decline (Francis et al., 2019). This is striking because worldwide consumption of nutrient-poor processed foods is increasing rapidly, and could play a role in accelerating cognitive decline (Harriden et al., 2022). Also, research suggests that cognitive difficulties in young adults co-occur with mental health complaints (Castaneda et al., 2008), and even mild cognitive impairment has been associated with reduced psychological wellbeing (Gates et al., 2014). Thus, snacking on processed foods, FVI and overall diet quality could be modifiable targets to reduce cognitive and mental health complaints in young adults as they begin, and ultimately, to slow age-related deterioration.

The nature of the relationship between dietary intake and psychological health may differ depending on how it is studied (prospectively or cross-sectionally), the timeframe of investigation, and whether the contribution of specific dietary components is evaluated. Measuring the impact of whole diet represents the cumulative and interactive effects of multiple dietary components (Cespedes & Hu, 2015; Hu, 2002; Naska et al., 2017). However, few studies have evaluated the impact of the foods central to a healthful dietary pattern, alongside overall diet quality to identify and compare the most potent, effective approach for enhancing psychological health (Collins et al., 2022). Research suggests that FVI may have a unique contribution to psychological health, but replication of current studies is required (Rooney et al., 2013). Study two and three of this thesis will extend the current literature by addressing the need for replication and prospective data, as identified by the narrative and systematic reviews. Additionally, by exploring the relationships over several months, this will allow for an evaluation of how early the relationships emerge (a question raised by the previous systematic review in Chapter 2), and for how long they hold for.

Thus, the following studies aimed to explore the relationship between consumption of nutrient-rich or nutrient-poor foods, overall diet quality and psychological health, both cross-sectionally (study 2) and prospectively (study 3). The independent associations of the frequency and quantity of FVI, frequent snacking on energy-dense, processed foods, and the role of cognitive failures as a potential mediator were explored to replicate the previous cross-sectional study within a university student population. Then, the relationships were measured and analysed over time, which builds on study 1 (in Chapter 3) and study 2, as study 3 includes measurement across three time points for a total of four months. The direct and indirect relationship between dietary intake, overall diet quality and depression, anxiety, stress, and wellbeing were investigated.

It was predicted that higher diet quality scores would be associated with increased psychological health (reduced symptoms of depression, anxiety, and stress, but increased wellbeing), both cross-sectionally, and over time. Furthermore, greater nutrient-rich FVI (as separate frequency and portions variables) would be associated with increased psychological health, whereas greater nutrient-poor sweet and savoury snacking would be associated with decreased psychological health. It was also predicted that cognitive failures would mediate the relationship between dietary intake or diet quality and psychological health (both cross-sectionally and prospectively), whereby higher FVI (quantity and frequency) and overall diet quality would negatively predict cognitive failures, and thus increase psychological health (reduce ill-being and increase wellbeing), but frequent sweet and savoury snacking would positively predict cognitive failures, and thus reduce psychological health.

4.2 Study 2 Methods

Participants

Participants were recruited through the Research Participation Scheme (RPS) at Aston University and the University of Birmingham in the UK, they received course credits in exchange for their time. A total of 452 University undergraduate students provided informed consent to take part, with 379 participants completing this online cross-sectional study. Participants completed an initial screener questionnaire to ensure they met the inclusion criteria. Participants who rated their general health as poor over the last 12 months, who currently have or had diabetes, or an eating disorder and/or medically diagnosed food allergy, high blood pressure, a heart attack, or were experiencing medical illness were not eligible to participate (50 participants did not meet the screening criteria and 23 eligible participants left the survey incomplete). The study was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample Size

Using G*Power, with alpha set at 0.05, modelling a small effect size, and power at 80%, the minimum required sample size was 395 participants (Faul et al., 2009). However, to account for participants who might not complete the study in full, or whose data may need to be excluded from analysis, we aimed to recruit a minimum of 450 participants to the study which was achieved.

Design

This is a cross-sectional design; participants completed measures of demographic, health, and lifestyle behaviour, eating behaviour, dietary intake, mental health, psychological wellbeing, and cognition, at one point in time via an online platform (www.gorilla.sc).

Measures

The aforementioned measures of lifestyle behaviours, health and demographic information, dietary intake, psychological health, and cognition from study 1 (Chapter 3) were applied here again, except for the online Stop-Signal Task, because based on comparisons with the literature, the task design was not challenging enough to identify valid inhibitory control scores (Padilla et al., 2013). In addition, the Short-form Food Frequency Questionnaire (SF-FFQ) was used to calculate overall diet quality represented by five food components: fruit intake, vegetable intake, oily fish consumption, fat consumption and non-milk extrinsic sugars (NMES). NMES derive from processed foods as they are not naturally found in the cellular structure of food. A score between 1 and 3 is allocated for each food component, 3 indicates that the UK dietary recommendations have been met for that food group. The overall minimum possible score is 5, the maximum 15. A score of 12 or above indicates a healthy dietary pattern (Cleghorn & Cade, 2016). The following individual food group consumption (frequency) in a "typical" week, over the past month and portions per day were also investigated in this study: (1) fruit consumption, which included fresh or tinned; (2) vegetable consumption, which included fresh, tinned, or frozen, but not potatoes; (3) sweet snacking, which included biscuits, cakes, chocolate, and sweets, and; (4) savoury snacking, which included crisps or savoury snacks. Therefore, the SF-FFQ was used to evaluate the extent to which a participant conforms to the dietary recommendations (diet quality score), while analysing intake (frequency and quantity) of the core food components that contribute to good diet quality. Previous studies have highlighted the need of a brief, validated measure of diet quality to be used in studies involving young adults (Jacka et al., 2011). The SF-FFQ has been suggested as a solution to this, as it may maximise student engagement, compared to longer and more laborious FFQs.

Procedure

The Gorilla Experiment platform (www.gorilla.sc) was used to create and host the cross-sectional study. The survey was completed by participants online using a computer; phones or tablets were not permitted. Participants who expressed a willingness to take part using the RPS provided informed consent and were screened for the inclusion criteria. Eligible participants then progressed through the series of questionnaires measuring demographic information, dietary intake, lifestyle behaviours, psychological health, cognition, mood, and appetite, and were finally debriefed.

Statistical analyses

Descriptive data were calculated as means with standard deviation, or as frequencies (%) for categorical variables. For the frequency of dietary intake, the median and range is presented. Simple linear and stepwise multiple regression analyses were conducted to explore the relationship between dietary intake and psychological health (before and after including the covariates: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake). The selection of these covariates was informed by previous literature (Gibson-Smith et al., 2020; Ocean et al., 2019; Mihrshahi et al., 2015). The stepwise analysis put predictors into competition with each other by applying the ≤ 0.050 to enter and ≥ 0.1 to remove criteria. Separate models were conducted for dietary intake (food frequency) for fruit, vegetable, sweet and savoury snack consumption as predictors. Fruit and vegetable intake (separately) quantified as portions consumed were also explored as separate predictors. The outcome variables included four different measures of psychological health: symptoms of depression, anxiety, stress, and positive psychological wellbeing scores. Multiple mediation analyses were performed using PROCESS, version 3.5 (Hayes., 2017) in SPSS version 26 using bootstrapping over 5,000 samples. The mediator included in the models was cognitive failures score. All mediation models included covariates that were consistently, significantly associated with the psychological health outcomes at the $p < 0.01$ level, which were general health rating and exercise (this replicates the mediation paths shown in Chapter 3, Figure 3.1).

4.3 Study 2 Results

4.3.1 Descriptive Results

***Participant characteristics*:** Descriptive statistics regarding demographic and lifestyle behaviour information for the University student sample are presented in Table 4.1. Ninety percent of the sample were female and 55.7% identified as white for ethnicity. The mean BMI for the sample was 22.5 (SD =4.4) and 67.6.% were a healthy weight. On average, participants exercised for 2.7 hours per week and most of the sample rated their general health as good to excellent (93%). Furthermore, 90.8% had never smoked and 82% consumed less than 14 units of alcohol per week or rarely/never drink.

Table 4.1 Sample demographic information and lifestyle behaviour (n = 379).

Characteristics	***N/Mean(SD)***	**Percentage/range**
Mean age in years (SD)	19.0 (1.5)	17-33
Sex		
Female	341	90.0
Male	38	10.0
Ethnicity/Race		
White	211	55.7
Asian	114	30.1
Black	28	7.4
Chinese	7	1.8
Mixed	7	1.8
Other	12	3.2
Mean body mass index (BMI)	22.5 (4.4)	14-55
Underweight	56	14.8
Healthy weight	256	67.6
Overweight	45	11.8
Obese	22	5.8
Mean exercise (total minutes) (SD)	164.3 (141.4)	0-1080
General health rating		
Excellent	69	18.2
Very good	181	47.8
Good	102	26.9
Fair	27	7.1
Smoking		
Never smoked > 100 cigarettes	344	90.8
Current smoker	21	5.5
Ex-smoker	14	3.7
Alcohol intake (units per week)		
Rarely or never drink	168	44.3
< 14 units	143	37.7
14-21 units	56	14.8
> 21 units	12	3.2
Eating style (TFEQ)		
Uncontrolled eating score	2.3 (0.6)	1.2-3.9
Cognitive restraint score	2.2 (0.6)	1-3.8
Emotional eating score	2.2 (0.7)	1-4.0

Note: *SD* = standard deviation; NHS classifications for BMI ranges were used; Underweight < 18.5, Healthy weight 18.5-24.9, Overweight 25-29.9, Obese ≥ 30; Eating style (TFEQ) average scores for each dimension (uncontrolled eating, cognitive restraint, and emotional eating) indicate a non-disordered sample.

Dietary intake: The frequency of dietary intake showed that on average, sweet, savoury snacks and fruit were each consumed 2-3 times a week, while vegetables were consumed 4-6 times a week. The range of responses was from rarely or never to 5+ times a day; overall participants were not consuming fruit and vegetables frequently enough to reach recommended intake. On average, participants consumed 1.8 portions of fruit per day (SD = 1.2) and 2.0 portions of vegetables per day (SD = 1.4), thus combined consumption was short of the recommended daily intake. Fruit intake ranged from 0 to 9 portions per day and vegetable intake ranged from 0 to 10 portions per day. In addition, the mean diet quality score (Mean = 9.7, SD = 1.6) indicated that overall, the student sample's diet could not be classified as following a healthy dietary pattern (a score greater than 12). Ten percent of the sample identified as vegetarian or vegan.

Cognition: For cognitive failures scores, the average frequency, variance, and range of cognitive failures scores were as follows: mean = 43.8, SD = 14.4, range = 7-86. These statistical values are similar to other University student samples (Ekici et al., 2016; Dzubur et al., 2020).

***Psychological health*:** Descriptive statistics for mood ratings and psychological health are presented in Table 4.2, below. VAS scores show that on average participants were happy and alert. Overall, mean depression and anxiety scores were considered non-clinical, anxiety scores were mild and stress levels were moderate (Andreou et al., 2011; Bjelland et al., 2002). The mean psychological wellbeing score (46.0) is similar to other UK general population groups (Bartram et al., 2011) including our previous study using this measure.

Table 4.2 Descriptive results for mood ratings and psychological health (n = 379).

Outcome	**Mean (SD)**	**Range**
Sad (VAS)	30.7 (25.3)	0-88
Happy (VAS)	60.4 (20.1)	0-100
Anxious (VAS)	30.8 (26.3)	0-100
Alert (VAS)	57.7 (23.0)	0-100
Drowsy (VAS)	45.9 (25.4)	0-100
Withdrawn (VAS)	34.6 (26.9)	0-99
Hungry (VAS)	39.4 (30.4)	0-100
Depression score (HADS)	5.8 (2.7)	1-15
Anxiety score (HADS)	8.7 (4.0)	0-20
Stress score (PSS)	20.3 (6.3)	5-35
Psychological wellbeing (WEMWBS)	46.0 (7.9)	21-66

4.3.2 Regression Results

Simple linear regression: Prior to the inclusion of covariates, simple linear regression was conducted to analyse fruit and vegetables (frequency or portions), overall diet quality and sweet and savoury snacking consumed as predictors of either depression, anxiety, stress, or wellbeing scores. The simple linear regression results are presented in Table 4.3, below. Frequency of fruit and vegetable consumption and portions of vegetables consumed negatively predicted depression scores (β = -.149, β = -.106, β = -.133, *ps* < 0.05). Frequency of vegetable consumption and portions of vegetables consumed positively predicted psychological wellbeing (β = .115, β = .155, *ps* < 0.05). The results also revealed that portions (but not frequency) of fruit consumption positively predicted psychological wellbeing scores (β = .115, p < 0.05). In addition, sweet and savoury snacking negatively predicted psychological wellbeing scores (β = -.150, β = -.131, *ps* < 0.05). Furthermore, sweet, and savoury snacking positively predicted stress scores (β = .192, β = .194, *ps* < 0.001). Diet quality did not predict any of the psychological health outcomes.

Table 4.3 Simple regression results for the relationship between snacking, fruit, vegetable intake or diet quality and psychological health (n = 379).

Depression	***Fruit frequency***	***Vegetable frequency***	***Fruit portions***	***Vegetable portions***	***Diet quality***	***Sweet snacking***	***Savoury snacking***
Unstandardised beta	-.230	-.184	-.101	-.257	-.094	.060	.094
Standardised beta	-.149	-.106	-.045	-.133	-.055	.033	.053
p value	.004	.039	.381	.010	.287	.517	.299
R^2	.022	.011	.002	.018	.003	.001	.003
Anxiety	***Fruit frequency***	***Vegetable frequency***	***Fruit portions***	***Vegetable portions***	***Diet quality***	***Sweet snacking***	***Savoury snacking***
Unstandardised beta	-.101	-.109	-.003	-.023	-.220	.230	.044
Standardised beta	-.044	-.042	-.001	-.008	-.086	.085	.017
p value	.397	.417	.986	.876	.095	.098	.743
R^2	.002	.002	.000	.000	.007	.007	.000
Stress	***Fruit frequency***	***Vegetable frequency***	***Fruit portions***	***Vegetable portions***	***Diet quality***	***Sweet snacking***	***Savoury snacking***
Unstandardised beta	-.354	-.259	-.367	-.192	-.055	.817	.803
Standardised beta	-.098	-.063	-.070	-.042	-.014	.192	.194
p value	.057	.220	.177	.414	.790	<.001	<.001
R^2	.010	.004	.005	.002	.000	.037	.038
Wellbeing	***Fruit frequency***	***Vegetable frequency***	***Fruit portions***	***Vegetable portions***	***Diet quality***	***Sweet snacking***	***Savoury snacking***
Unstandardised beta	.430	.595	.764	.891	.058	-.803	-.684
Standardised beta	.094	.115	.115	.155	.011	-.150	-.131
p value	.068	.025	.026	.002	.825	.004	.011
R^2	.009	.013	.013	.024	.000	.022	.017

Note: Separate simple linear regression models were analysed for sweet and savoury snacking, fruit and vegetable frequency or portions consumed and diet quality as predictors of depression, anxiety, stress, and wellbeing scores (prior to the inclusion of covariates in stepwise regression models shown in Tables 4-6).

Stepwise regression: To evaluate the relative contribution of dietary intake (frequency and portions of fruit and vegetable consumption, sweet and savoury snacking) and overall diet quality as predictors of psychological health, compared to other known predictors, the following covariates were included in a stepwise multiple regression: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake. The significant stepwise regression models' beta values, *p* values, change in R^2, and the adjusted R^2 for each step of the analysis are presented in Tables 4.4-4.6. The frequency of vegetable consumption and overall diet quality were not retained as significant predictors in any of these cross-sectional stepwise models.

The significant stepwise models for depression scores are presented in Table 4.4. The first model selected fruit frequency for entry first (β = -.146, p = 0.004, R^2 = .022), then BMI was added (β = .135, p = 0.008, R^2 = .041), followed by smoking status (β = .138, p = 0.007, R^2 = .054), and finally alcohol consumption (β = -.118, p = 0.021, R^2 = .067). Overall, the model predicted 5.7% of the variance in depression scores (F (4, 374) = 6.714, p < 0.001, adjusted R^2 total = .057). The second model selected vegetable portions for entry first (β = -.106, p = 0.036, R^2 = .018), then BMI was added (β = .118, p = 0.020, R^2 = .031), followed by smoking status (β = .139, p = 0.007, R^2 = .044), and finally alcohol consumption (β = -.115, p = 0.026, R^2 = .057). Overall, the model predicted 4.7% of the variance in depression scores (F (4, 374) = 5.642, p < 0.001, adjusted R^2 total = .047).

Table 4.4 The significant stepwise regression models for the relationship between fruit frequency and vegetables portions with depression, including covariates (n = 379).

Depression	***Fruit frequency***	***BMI***	***Smoking status***	***Alcohol consumption***
Unstandardised beta	-.255	.082	.758	-.388
Standardised beta	-.146	.135	.138	-.118
p value	.004	.008	.007	.021
	Step 1	*Step 2*	*Step 3*	*Step 4*
R, R^2	.149, .022	.202, .041	.232, .054	.259, .067
R^2 change	.022	.018	.013	.013
Adjusted R^2	.020	.036	.046	.057
Depression	***Vegetable portions***	***BMI***	***Smoking status***	***Alcohol consumption***
Unstandardised beta	-.205	.071	.765	-.378
Standardised beta	-.106	.118	.139	-.115
p value	.036	.020	.007	.026
	Step 1	*Step 2*	*Step 3*	*Step 4*
R, R^2	.133, .018	.176, .031	.211, .044	.239, .057
R^2 change	.018	.013	.013	.013
Adjusted R^2	.015	.026	.037	.047

Note: Depression: Step 1 fruit frequency or vegetable portions were entered in separate models; Step 2 BMI was added; Step 3 smoking status added; Step 4 alcohol consumption added.

The significant stepwise models for wellbeing scores are presented in Table 4.5. All four models selected general health rating for entry first (β = .252-.261, p < 0.001, R^2 = .070) followed by either sweet snacking (*β* = -.126, *p* = .011, R^2 = .086), savoury snacking (*β* = -.108, *p* = .031, R^2 = .081), fruit portions (*β* = .108, *p* = .029, R^2 = .081) or vegetable portions (*β* = .134, *p* = 0.007, R^2 = .088). Overall, the model for vegetable portions including general health rating predicted the largest amount of variance (8.3%) in wellbeing scores (*F* (2, 376) = 18.065, *p* < 0.001, adjusted R^2 total = .083) followed by sweet snacking (*F* (2, 376) = 17.596, *p* < 0.001, adjusted R^2 total = .081; 8.1%), fruit portions (*F* (2, 376) = 16.668, *p* < 0.001, adjusted R^2 total = .077; 7.7%) and finally savoury snacking (*F* (2, 376) = 16.624, *p* < 0.001, adjusted R^2 total = .076; 7.6%). After including the covariates in the stepwise regression, frequency of vegetable consumption was no longer a significant predictor of depression or wellbeing scores (shown in the simple regression results).

Table 4.5 The significant stepwise regression models for the relationship between sweet and savoury snacking, fruit, and vegetable portions with wellbeing, including covariates (n = 379).

Wellbeing	***General health rating***	***Sweet snacking***
Unstandardised beta	2.427	-.679
Standardised beta	.252	-.126
p value	<.001	.011
	Step 1	*Step 2*
R, R²	.264, .070	.293, .086
R² change	.070	.016
Adjusted R²	.067	.081
Wellbeing	***General health rating***	***Savoury snacking***
Unstandardised beta	2.443	-.562
Standardised beta	.254	-.108
p value	<.001	.031
	Step 1	*Step 2*
R, R²	.264, .070	.285, .081
R² change	.070	.011
Adjusted R²	.067	.076
Wellbeing	***General health rating***	***Fruit portions***
Unstandardised beta	2.512	.720
Standardised beta	.261	.108
p value	<.001	.029
	Step 1	*Step 2*
R, R²	.264, .070	.285, .081
R² change	.070	.012
Adjusted R²	.067	.077
Wellbeing	***General health rating***	***Vegetable portions***
Unstandardised beta	2.432	.771
Standardised beta	.253	.134

p value	<.001	.007
	Step 1	*Step 2*
R, R^2	.264, .070	.296, .088
R^2 change	.070	.018
Adjusted R^2	.067	.083

Note: Wellbeing: Step 1 general health rating was entered for all models; Step 2 sweet or savoury snacking, fruit or vegetable portions were added in separate models.

The significant stepwise models for stress scores are presented in Table 4.6. Two models emerged that both selected general health rating for entry first (β = -.263-.262, p < 0.001, R^2 = .077) followed by either sweet snacking (β = .168, p = .001, R^2 = .105) or savoury snacking (β = .170, p = .001, R^2 = .106). Both models predicted 10% of the variance in stress scores: savoury snacking (F (2, 376) = 22.269, p < 0.001, adjusted R^2 total = .101) and sweet snacking (F (2, 376) = 22.092, p < 0.001, adjusted R^2 total = .100). There were no significant stepwise models for anxiety (*ps* > 0.05).

Table 4.6 The significant stepwise regression models for the relationship between sweet and savoury snacking with stress, including covariates (n = 379).

Stress	***General health rating***	***Sweet snacking***
Unstandardised beta	-2.00	.714
Standardised beta	-.263	.168
p value	<.001	.001
	Step 1	*Step 2*
R, R^2	.278, .077	.324, .105
R^2 change	.077	.028
Adjusted R^2	.075	.100
Stress	***General health rating***	***Savoury snacking***
Unstandardised beta	-1.998	.703
Standardised beta	-.262	.170
p value	<.001	.001
	Step 1	*Step 2*
R, R^2	.278, .077	.325, .106
R^2 change	.077	.029
Adjusted R^2	.075	.101

Note: Stress: Step 1 general health rating was entered for both models; Step 2 sweet or savoury snacking were added in separate models.

Focussing on dietary intake within these models, the results showed that fruit frequency independently contributes 2.2% to the variance explained in depression scores (R^2 change at step 1 = .022). Whereas vegetable portions independently contribute 1.8% to the variance explained in depression scores (R^2 change at step 1 = .018). Furthermore, vegetable portions independently contribute 1.8% to the variance explained in wellbeing scores (R^2 change at step 2 = .018). Whereas fruit portions (but not frequency) independently contribute 1.2% to the variance explained in wellbeing scores (R^2 change at step 2 = .012). Frequency of sweet and savoury snacking each independently contribute 1.6% and 1.1% to the variance explained in wellbeing scores (R^2 change at step 2 = .016 and .011, respectively). Additionally, frequency of sweet and savoury snacking each independently contribute 2.8% and 2.9% to the variance explained in stress scores (R^2 change at step 2 = .028 and .029, respectively).

4.3.3 Mediation Results

Mediation analyses were applied to assess all models of dietary intake and quality with cognitive failures as the mediator, and the outcomes variables: depression, anxiety, stress, and positive wellbeing scores. The mediation models included general health rating and exercise as covariates.

Cognitive failures mediated the relationship between savoury snacking and psychological health whilst controlling for general health rating and exercise. Specifically, savoury snacking significantly positively predicted cognitive failures (*a* pathway; $B = 1.03$, $p < .05$). As cognitive failures increased, so did depression (*b* pathway; $B = 0.06$, $p < 0.001$), stress (*b* pathway; $B = 0.12$, $p < 0.001$) and anxiety scores (*b* pathway; $B = 0.08$, $p < 0.001$), whereas wellbeing scores decreased (*b* pathway; $B = - 0.12$, $p < 0.001$). Savoury snacking was not a direct significant predictor of wellbeing, depression, or anxiety when cognitive failures were held constant (*c'* pathways, $p > 0.05$), indicating full mediation had occurred. However, savoury snacking was a direct significant predictor of stress ($p < 0.05$), indicating partial mediation had occurred here. Savoury snacking was a significant predictor of wellbeing and stress (*ps* < 0.05) but not depression or anxiety (*ps* > 0.05) when the indirect and direct pathways were combined (*c* total pathway). Further, examining the indirect effect (*ab* pathway) indicated that mediation had occurred for: depression (*ab* = .064, 95% CI [.005, .123]); anxiety (*ab* = .086, 95% CI [.007, .178]); stress (*ab* = .121, 95% CI [.012, .256]), and; wellbeing (*ab* = -.122, 95% CI [-.264, -.011]). No further significant mediation was identified for the other predictors of dietary intake or overall diet quality.

4.4 Study 3 Methods

Participants

Participants were recruited through the Research Participation Scheme (RPS), as per study 2. A total of 452 University undergraduate students provided informed consent to take part, with 183 participants completing this online prospective study (all three timepoints). The same inclusion criteria for study 2 was used here. Participants completed an initial screener questionnaire to ensure they met the inclusion criteria. Fifty participants did not meet the screening criteria and 23 eligible participants left the survey incomplete. Thus, three hundred and seventy-nine participants completed the study at baseline (timepoint 1, T1), of which 230 also took part 1 month later (timepoint 2, T2) and the final sample at 4 months (timepoint 3, T3) was 183 (after the removal of four outliers with clinical mental health scores). The study was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample size

Using G*Power, a criterion significance level of 0.05, modelling a small effect size, and power at 80%, a total of 395 participants were required across all three timepoints (Faul et al., 2009). However, we aimed to recruit a minimum of 450 participants to the study.

Design

This is a three-part, within-subjects, prospective design whereby participants completed measures at all three timepoints over the course of four months (October 2017, November 2017, and January 2018). Participants completed the following measures at each timepoint as part of an online survey through the Gorilla Experiment platform (www.gorilla.sc).

Measures

The same measures used in study 1 (Chapter 3) and the previously presented cross-sectional study (study 2) were used during this prospective study for lifestyle behaviours, health and demographic information, dietary intake, cognition, and psychological health.

Procedure

The Gorilla Experiment platform (www.gorilla.sc) was used to create and host the prospective study. Participants who expressed a willingness to take part using the RPS provided informed consent and were screened for the inclusion criteria. Eligible participants then progressed through the series of questionnaires measuring demographic information, dietary intake, lifestyle behaviours, psychological health, cognition, mood, and appetite, and were finally debriefed. Once the first timepoint was completed, participants were asked to enter their email address, and this generated a countdown to remind the participant precisely one month later to complete the study. After the second completion, participants were reminded to complete the study for a final time in January (two months later). Additional email reminders were sent to participants to reduce drop out.

Statistical analyses

Descriptive analyses were used to evaluate the sample's lifestyle behaviours, health, and demographic information, in addition to the dietary intake, diet quality, cognition and psychological health variables. For the frequency of dietary intake, the median and range is presented. Stepwise multiple regression was used to analyse the data, predictors were put into competition with each other by applying the ≤ 0.050 to enter and ≥ 0.1 to remove criteria. The predictor variables were baseline (T1) fruit and vegetable intake (portions and frequency), sweet and savoury snacking, and overall diet quality. The following covariates were included in the stepwise multiple regression: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake. Furthermore, each model included baseline psychological health score (e.g., T1 depression score was included when the outcome variable was T2/3 depression score). The outcome variables were psychological health at T2 and T3, including symptoms of depression, anxiety, stress, and positive wellbeing scores. Mediation analyses were performed using PROCESS, version 3.5 (Hayes., 2017) in SPSS version 26 using bootstrapping over 5,000 samples. The mediator included in the models was either T2 or T3 cognitive failures score (to correspond with the timepoint of the psychological health outcome). All mediation models included baseline (T1) psychological health score relevant to the outcome variable, with general health rating and exercise as covariates.

4.5 Study 3 Results

4.5.1 Descriptive Results

Participant characteristics: Baseline (T1) descriptive statistics regarding demographic and lifestyle behaviour information for the University student sample who completed all three timepoints (n = 183), are presented in Table 4.7. Ninety percent of the sample were female and 54.1% identified as white for ethnicity. The mean BMI for the sample was 22.6 (SD =4.5) and 62.3% were healthy weight. On average, participants exercised for 2.7 hours per week and most of the sample rated their general health as good to excellent (92%). Furthermore, 88.5% had never smoked and 84% consumed less than 14 units of alcohol per week or rarely/never drink.

Table 4.7 Sample demographic information and lifestyle behaviour (n = 183).

Characteristics	*N/Mean(SD)*	Percentage/range
Mean age in years (SD)	19.2 (1.7)	18-33
Sex		
Female	164	89.6
Male	19	10.4
Ethnicity/Race		
White	99	54.1
Asian	61	33.3
Black	12	6.6
Chinese	4	2.2
Mixed	3	1.6
Other	4	2.2
Mean body mass index (BMI)	22.6 (4.5)	14-39
Underweight	30	16.4
Healthy weight	114	62.3
Overweight	23	12.6
Obese	16	8.7
Mean exercise (total minutes)	164.2 (145.5)	0-1050
General health rating		
Excellent	30	16.4
Very good	88	48.0
Good	51	27.9
Fair	14	7.7
Smoking		
Never smoked > 100 cigarettes	162	88.5
Current smoker	13	7.1
Ex-smoker	8	4.4
Alcohol intake (units per week)		
Rarely or never drink	85	46.4
< 14 units	68	37.2
14-21 units	25	13.7
> 21 units	5	2.7
Eating style (TFEQ)		
Uncontrolled eating score	2.3 (0.6)	1.3-3.9
Cognitive restraint score	2.2 (0.6)	1.2-3.7
Emotional eating score	2.2 (0.7)	1-4.0

Note: *SD* = standard deviation; NHS classifications for BMI ranges were used; Underweight < 18.5, Healthy weight 18.5-24.9, Overweight 25-29.9, Obese ≥ 30; Eating style (TFEQ) average scores for each dimension (uncontrolled eating, cognitive restraint, and emotional eating) indicate a non-disordered sample.

Dietary intake and quality: Descriptive statistics for fruit and vegetable portions and psychological health are presented in Table 4.8. The average combined daily fruit and vegetable consumption (portions) did not meet the five a day recommendation. Fruit intake ranged from 0-5 portions a day across all three timepoints. Vegetable intake ranged from 0-6 portions a day at timepoints 1 and 3, but 0-7 portions at T2. In addition, the mean diet quality scores indicated that overall, the student sample's diet could not be classified as following a healthy dietary pattern (a score greater than 12). At baseline, 88% of the sample's diet quality score fell short of the UK dietary recommendations (scoring less than 12). The frequency of dietary intake showed that on average, vegetables were consumed 4-6 times a week at T1, fruit and vegetables were consumed 2-3 times a week across all other timepoints. Sweet snacks were consumed 4-6 times a week at T1, sweet and savoury snacks were consumed 2-3 times a week across all other timepoints. Frequency of consumption ranged from rarely or never to 5+ times a day across all timepoints for fruit and vegetable intake, sweet and savoury snacking. Five percent of the sample identified as vegetarian or vegan.

Table 4.8 Means and standard deviation (SD) of fruit and vegetable intake, diet quality and psychological health scores by timepoint (n = 183).

Mean (SD)	T1	T2	T3
Fruit portions	1.77 (1.08)	1.78 (1.10)	1.79 (1.15)
Vegetable portions	1.92 (1.23)	2.02 (1.35)	2.06 (1.32)
Diet quality	9.49 (1.96)	9.78 (1.80)	9.87 (1.83)
Cognitive failures	44.07 (14.47)	45.26 (14.12)	44.21 (14.61)
Depression score (HADS)	4.81 (3.23)	5.11 (3.73)	5.17 (3.70)
Anxiety score (HADS)	8.56 (4.14)	8.60 (4.23)	7.99, (4.13)
Stress score (PSS)	19.94 (6.70)	19.88 (6.73)	19.62 (6.92)
Psychological wellbeing (WEMWBS)	45.80 (8.09)	45.57 (8.46)	45.70 (8.94)

Cognition: Cognitive Failures scores ranged from 7 to 86 at T1, 15 to 87 at T2, and 10 to 92 at T3. The means and SD for each timepoint are presented in Table 4.8. On average, the scores were similar to other university student samples (Dzubur et al., 2020; Ekici et al., 2016).

Psychological health: Descriptive statistics for psychological health are presented in Table 4.8. On average, the depression and anxiety scores for the sample were considered non-clinical (Bjelland et al., 2002). Overall, the stress scores were indicative of moderate stress (Andreou et al., 2011). Additionally, mean psychological wellbeing scores are similar to other UK student population groups and ranged from 14 to 67 (Davoren et al., 2013).

4.5.2 Regression Results

Stepwise regression results: To evaluate the relative contribution of dietary intake (frequency and portions of fruit and vegetable consumption, sweet and savoury snacking) and overall diet quality (at T1) as predictors of psychological health (at T2 and T3), compared to other known predictors, the following covariates were included in a stepwise multiple regression: age, sex, BMI, exercise, general health rating, smoking status, and alcohol intake. In addition, T1 psychological health scores were included in the models according to the outcome variable. The significant stepwise regression models' beta values, *p* values, change in R^2, and the adjusted R^2 for each step of the analysis are presented in Tables 4.9 and 4.10.

There was one significant stepwise model for dietary intake predictors of psychological health at T2 (one month later), this is presented in 4.9, below. This model selected T1 anxiety scores for entry first (β = .669, p < .001, R^2 = .431), followed by sweet snacking (β = -.150, p = 0.007, R^2 = .453). Overall, the model predicted 44.7% of the variance in anxiety scores (F (2, 180) = 74.656, p < 0.001, adjusted R^2 total = .447). No further significant stepwise models at T2 were found.

Table 4.9 The significant stepwise regression model for the relationship between T1 sweet snacking and anxiety one month later (T2), including covariates (n = 183).

T2 Anxiety	***T1 Anxiety***	***T1 Sweet snacking***
Unstandardised beta	.683	-.420
Standardised beta	.669	-.150
p value	< .001	.007
	<u>*Step 1*</u>	<u>*Step 2*</u>
R, R^2	.657, .431	.673, .453
R^2 change	.431	.022
Adjusted R^2	.428	.447

Note: Anxiety: Step 1 T1 Anxiety score was entered, Step 2 Sweet snacking was added.
At T3 (month 4), sweet snacking no longer significantly predicted anxiety scores.

The significant stepwise models for dietary intake predictors of psychological health at T3 (month 4) are presented in Table 4.10. A significant stepwise model for depression scores was found, this selected T1 depression scores for entry first (β = .552, p < 0.001, R^2 = .328), followed by frequency of vegetable consumption (β = -.137, p = .025, R^2 = .346). Overall, the model predicted 33.9% of the variance in depression scores (F (2, 180) = 47.704, p < 0.001, adjusted R^2 total = .339). Furthermore, a significant stepwise model for stress scores was found, this selected frequency of fruit consumption for entry first (β = -.169, p = .025, R^2 = .033), followed by general health rating (β = -.161, p = .032, R^2 = .059). Overall, the model predicted 4.8% of the variance in stress scores (F (2, 180) = 5.295, p = 0.006, adjusted R^2 total = .048). Finally, a significant stepwise model for wellbeing scores was found, this selected T1 wellbeing scores for entry first (β = .577, p < 0.001, R^2 = .345) followed by frequency of fruit consumption (β = .126, p = .037, R^2 = .361). Overall, the model predicted 35.4% of the variance in wellbeing scores (F (2, 180) = 50.778, p < 0.001, adjusted R^2 total = .354). No further significant stepwise models at T3 were found. Fruit or vegetable portions, the frequency of savoury snacking, and diet quality score were not retained as significant predictors in any of the stepwise models.

Table 4.10 The significant stepwise regression models for the relationship between T1 frequency of fruit or vegetable consumption and T3 psychological health, including covariates (n = 183).

T3 Depression	***T1 Depression***	***T1 Vegetable frequency***
Unstandardised beta	.628	-.340
Standardised beta	.552	-.137
p value	<.001	.025
	Step 1	*Step 2*
R, R²	.573, .328	.589, .346
R² change	.328	.018
Adjusted R²	.324	.339
T3 Stress	***T1 Fruit frequency***	***General health rating***
Unstandardised beta	-.640	-1.332
Standardised beta	-.169	-.161
p value	.025	.032
	Step 1	*Step 2*
R, R²	.181, .033	.242, .059
R² change	.033	.026
Adjusted R²	.027	.048
T3 Wellbeing	***T1 Wellbeing***	***T1 Fruit frequency***
Unstandardised beta	.637	.635
Standardised beta	.577	.126
p value	<.001	.037
	Step 1	*Step 2*
R, R²	.587, .345	.601, .361
R² change	.345	.016
Adjusted R²	.341	.354

Note: Depression: Step 1 T1 Depression score was entered, Step 2 Vegetable frequency was added; Stress: Step 1 Fruit frequency was entered, Step 2 General health rating was added; Wellbeing: Step 1 T1 Wellbeing was entered, Step 2 Fruit frequency was added.

Focussing on dietary intake within these models, the results showed that sweet snacking was a significant negative predictor of T2 anxiety scores, which independently contributes 2.2% to the variance explained (R^2 change at step 2 = .022). Frequency of vegetable consumption was a significant negative predictor of T3 depression scores, which independently contributes 1.8% to the variance explained (R^2 change at step 2 = .018). Furthermore, frequency of fruit consumption was a significant negative predictor of T3 stress scores, which independently contributes 3.3% to the variance explained (R^2 change at step 1 = .033). In addition, frequency of fruit consumption was a significant positive predictor of T3 wellbeing scores, which independently contributes 1.6% to the variance explained (R^2 change at step 2 = .016). The unstandardised beta values (presented in Table 4.9 and 4.10) show that for every 1 unit increase in the frequency of sweet snacking, vegetable, or fruit consumption (e.g., from 4–6 times a week to 1–2 times a day), anxiety scores decrease by

.420 after one month, whereas depression scores decrease by .340, stress scores decrease by .640, while positive wellbeing scores increase by .635, by the fourth month. The adjusted, standardised beta estimates (after adjusting for the covariates retained in the stepwise models using hierarchical regression) were: -.072 (sweet snacking and T2 anxiety), -.137 (vegetable frequency and T3 depression), -.138 (fruit frequency and T3 stress) and .126 (fruit frequency and T3 wellbeing).

4.5.3 Mediation Results

Mediation analyses were applied to assess all models of dietary intake and overall diet quality with cognitive failures as the mediator, and the outcomes variables: depression, anxiety, stress, and positive wellbeing scores. The mediation models included baseline psychological health (e.g., T1 depression scores when analysing the outcome variable of T2/T3 depression), general health rating and exercise as covariates.

Cognitive failures mediated the relationship between T1 diet quality and T3 (month 4) stress scores (no significant mediation for depression, anxiety or wellbeing scores was found). This mediation did not occur after one month. Specifically, T1 overall diet quality significantly negatively predicted T3 cognitive failures (*a* pathway; $B = -1.39$, $p < .05$). As T3 cognitive failures increased, so did T3 stress scores (*b* pathway; $B = 0.20$, $p < .001$). Diet quality was not a direct significant predictor of stress scores at T3 when cognitive failures were held constant (*c'* pathway $p > 0.05$). Diet quality was not a significant predictor of T3 stress scores when the indirect and direct pathways were combined (*c* total pathway; $p > 0.05$). Further, examining the indirect effect (*ab* pathway) indicated that full mediation had occurred ($ab = -.28$, 95% CI [-.54, -.053]). No further mediation occurred for the other dietary intake predictors.

4.6 Summary of the Results across studies 1-3

To synthesise the results across the two cross-sectional and one prospective study, the following Table 4.11, provides a summary of the significant direct relationships observed for dietary intake and psychological health.

Table 4.11 A summary of the significant direct relationships observed across the three previous studies (within Chapters 3 & 4) for fruit and vegetable intake, sweet and savoury snacking.

Study 1 (cross-sectional) **n = 428**	**Study 2 (cross-sectional)** **n = 379**	**Study 3 (prospective)** **n = 183**
Fruit frequency and depression (negative direction) Fruit frequency and positive psychological wellbeing (positive direction)	Fruit frequency and depression (negative direction) Fruit portions (not frequency) and positive psychological wellbeing (positive direction)	Fruit frequency and stress (after four-months, negative direction, not depression) Fruit frequency and positive psychological wellbeing (after four-months, positive direction)
No relationship between vegetables and psychological health	Vegetable portions and depression (negative direction) Vegetable portions and positive psychological wellbeing (positive direction)	Vegetable frequency and depression (after four-months, negative direction)
Savoury snacking and anxiety (positive direction)	Savoury snacking and stress (positive direction) Savoury snacking and positive psychological wellbeing (negative direction)	No relationship between savoury snacking and psychological health
No relationship between sweet snacking and psychological health	Sweet snacking and stress (positive direction) Sweet snacking and positive psychological wellbeing (negative direction)	Sweet snacking and anxiety (after one-month, negative direction)

Note: The mediation results are not summarised here.

4.7 Discussion

These studies assessed the cross-sectional and prospective relationships between dietary intake, overall diet quality and psychological health, including the consumption of nutrient-poor sweet and savoury snacks, and nutrient-rich F&V, separately, in a student population. Additionally, these studies replicate and extend the previous study (study 1) investigating the indirect relationship between dietary intake and psychological health via cognitive failures, by assessing this again cross-sectionally, and in addition, prospectively. A summary of the direct relationships observed across studies 1-3 thus far in the thesis, is provided by Table 4.11, above.

Cross-sectionally, the significant associations observed in study 2 were for higher vegetable intake (portions not frequency) and reduced symptoms of depression. In addition, both fruit and vegetable portions positively predicted psychological wellbeing. Previous research has observed associations between higher quantities of F&V (portions), reduced symptoms of depression, higher happiness, and wellbeing (Lai et al., 2014; Lesani et al., 2016; Peltzer & Pengpid, 2017; Rooney et al., 2013). Furthermore, a significant, negative relationship between frequency of fruit consumption and symptoms of depression was observed cross-sectionally. This replicated the previous cross-sectional findings (study 1, Chapter 3) and previous research which has identified a link with reductions to distress indicative of depressive status (Kingsbury, 2015; Liu et al., 2007). Moreover, frequent sweet and savoury snacking negatively predicted psychological wellbeing and positively stress scores; these results support previous research highlighting the detrimental associations between nutrient-poor snacking, wellbeing, and stress in university students (El Ansari et al., 2014; Smith & Rogers, 2014). However, an association with increased symptoms of depression has also been reported (El Ansari et al., 2014), which was not observed in the current cross-sectional results.

Prospectively, frequency of fruit consumption at timepoint 1 (baseline) did not predict depression scores at timepoint 2 (one month), or at timepoint 3 (by four months) within study 3. This may be due to the shorter timeframe of observation compared to previous research which have investigated the relationship over 2-15 years (Dharmayani et al., 2021, 2022; Mihrshahi et al., 2015). At timepoint 1 (baseline) frequency of vegetable consumption negatively predicted symptoms of depression at timepoint 3 (by four months, but not one month later at T2). A previous UK prospective study observed that frequency of vegetable consumption had a larger effect on mental health compared to frequent consumption of fruit (Ocean et al., 2019). This supports the notion that vegetables may exert more potent effects than fruit for this aspect of mental health (Tuck et al., 2019, Chapter 2), and that over time the focus on frequency (how often) instead of daily portions may be required to sustain positive outcomes associated with habitual consumption. However, frequency of vegetable consumption did not predict perceived stress, despite previous evidence that following a vegan diet reduced stress levels (though frequency or intake of vegetables was not explicitly quantified in this study - Lee et al., 2019).

Frequency of fruit consumption significantly, positively predicted psychological wellbeing at timepoint 3 (month 4), not one month later at timepoint 2; this was not found cross-sectionally in study 2 (portions did instead), however, this association was observed in study 1 (see the aforementioned summary Table 4.11). Additionally, timepoint 1 (baseline) frequency of fruit consumption negatively predicted stress scores at timepoint 3 (at month 4). Therefore, for promoting positive psychological wellbeing and reducing stress levels over time, the results suggest that frequency of fruit consumption could be more important than vegetables. Previous research in university students has also observed positive effects of increasing the frequency of fruit consumption on reductions in perceived stress (El Ansari et al., 2014; Liu et al., 2007), and increases to positive psychological wellbeing (Conner et al., 2017). Moreover, studies in young adults (aged 17-25 years old) have observed that eating F&V 'daily' or 'almost daily' was associated with higher scores of subjective happiness compared to those consuming F&V less frequently (Piqueras et al., 2011). This further emphasises the importance of comparing the links between F&V quantity and frequency with a range of psychological health measures.

After the inclusion of covariates, cross-sectionally, cognitive failures mediated the relationship between savoury snacking and all outcomes of psychological health (depressions, anxiety, stress, and wellbeing scores), whereas cognitive failures did not mediate the relationship between either FVI or sweet snacking and psychological health. These findings reflect the results of the previous study in Chapter 3 (study 1) and suggest a specific, cross-sectional indirect relationship between savoury, nutrient-poor snack intake and psychological health, via cognitive failures may exist. These novel indirect findings support the literature that has observed an association between nutrient-poor food intake and reduced cognitive performance (Attuquayefio & Stevenson, 2015; Beilharz et al., 2015; Francis & Stevenson, 2011), and, that cognitive impairment is linked to reduced psychological health (Gates et al., 2014). However, this mediation was not observed prospectively in study 3, after one month (T2) or by the fourth month (T3).

Cognitive failures did mediate the relationship between timepoint 1 (baseline) overall diet quality scores and self-reported stress at timepoint 3 (at month four, but not after one month). This supports previous research by highlighting a relationship between optimal diet quality, better cognitive performance, and lower perceived stress (Young et al., 2020). Improvements to overall diet quality over time may reduce the risk of memory errors that have been linked to increased stress levels (Boals & Banks, 2012). No further direct relationships were found between overall diet quality scores and psychological health, which is surprising considering the previous evidence showing a link with depression (Lai et al., 2013; Quirk et al., 2013). However, this study did not classify participants as depressed or not, and previous research observes the relationship over a longer duration than the course of four months (Sánchez-Villegas et al., 2015). This may explain why only an indirect link between diet quality and stress emerged.

The current null findings also align with a previous large prospective study accounting for covariates, that reported a non-significant relationship between overall diet quality and depression risk (Chocano-Bedoya et al., 2013). Both this and the current prospective study accounted for psychological scores at baseline to address the issues of reverse causation, which may explain the significant results seen in other studies that fail to do so. Baseline psychological health is likely to have an impact on the relationships observed but is often not accounted for in research (Mujcic & Oswald; Brookie, 2018). Also, positive outcomes to psychological health tend to involve participants with higher baseline levels of poor physical health (Pomerleau et al., 2005), and the current studies recruited healthy adults and covaried for general health rating, which may attenuate the relationship between overall diet quality and psychological health. Furthermore, the average student diet quality scores did not reach the threshold for being classified as following a healthy dietary pattern, which may limit comparisons with previous studies that involve interventions that increase diet quality (O'Neill et al., 2022).

For anxiety scores, only sweet snacking negatively (unexpectedly) predicted stress scores one month later (timepoint 2), but this did not continue over the course of four months (at timepoint 3). Habitual nutrient-poor processed food intake is consistently linked with higher anxiety (Collins et al., 2022; Jacka et al., 2011; Phillips et al., 2018), which is the opposite direction to that was observed. However, the current finding may be explained by how sweet snacking in the young adult population is often used as a coping mechanism for lowering the presence of anxious emotions (Caso, Miriam, Rosa, & Mark, 2020). No other significant models emerged at timepoint 2 (none for savoury snacking or F&V, or overall diet quality). At timepoint 3 (four months), no significant models emerged for snacking (only for frequency of FVI). Therefore, the current results provide no evidence for direct links between nutrient-poor snacking and psychological health over time past one month. Furthermore, no relationship between FVI and anxiety was found. This supports previous null associations between F&V consumption and anxiety (Jacka et al., 2010; Oellingrath et al., 2014; Yannakoulia et al., 2008).

Principally, there is some reliability in the findings across the cross-sectional studies. For instance, the associations between F&V portions and psychological health may be more readily observed cross-sectionally, whereas sustained high intake of F&V (portions per day) may be essential for observing the influence prospectively (Conner et al., 2017; De Leon et al., 2022). The current sample means suggest that F&V intake was relatively stable over timepoints 1-2 (means ranged from 1.77-2.06 portions for each, with combined intake <5 portions a day), however, the means may conceal larger shifts, so analysis beyond mean levels may be required (e.g., trajectories of intake and psychological health). However, eating behaviour has been shown to remain stable within intervals of less than a year, with change in healthy food intake shown to be minimal, even across seasons (Henderson et al., 2023; Schätzer et al., 2009). Overall, frequent consumption of fruit and vegetables may exert a positive, prospective influence on psychological health over time (fruit on wellbeing and stress, vegetables on depression at four months), which could present a simple and effective method for enhancing and protecting it. A recent study explored a range of biopsychosocial factors (sociodemographic, psychosocial, emotional, cognitive, and lifestyle) to predict psychological wellbeing over one year (Chilver et al., 2023). Indeed, the strongest lifestyle predictors were exercise, and fruit and vegetable consumption. Frequent consumption of F&V may contribute to psychological wellbeing through sufficient intake of mood-related micronutrients that reduce the risk of poor mental health over time (Rooney et al., 2013).

Strengths of these studies included that they involved data collected across two UK universities. Additionally, aspects of dietary intake were analysed alongside a measure of overall diet quality, while allowing comparisons to be made for cross-sectional and prospective relationships. Different assessment methods of dietary intake and psychological health may contribute to the current conflicting results in previous research (Dharmayani et al., 2021), however a strength of the current studies is that the same SF-FFQ was used to assess both dietary intake and quality. Additionally, Solomou and colleagues (2022) called for the use of a brief, validated measure of dietary intake and quality to maximise engagement in university student samples (with the short-form Food Frequency Questionnaire SF-FFQ being given as an example measure), and this has been applied in the current and previous cross-sectional study. Study 2 combined with study 1 (Chapter 3) – which it replicates - aimed to evaluate the relationships using consistent methodologies, while comparing frequency and quantity of F&V consumption to clarify variations in previous results, as previous research has focussed on portions or meeting national guidelines (Collins et al., 2022). The results emphasise that assessing frequency of consumption may be relevant prospectively. Furthermore, the prospective data supports the temporal order observed in previous studies; that dietary intake impacts psychological health over time (Jacka et al., 2015; White et al., 2013). In addition, this study measured and included a range of demographic and lifestyle variables within the analysis, however, students' income was not assessed, and this is known to impact psychological health (Marmot et al., 1997). Further longitudinal studies in young adult populations will enhance our understanding of whether whole dietary approaches (compared with components such as snacking or FVI) can effectively protect cognition, and in turn promote psychological health. More prospective (short-term and long-term) studies, combined with experimental research that induces change in F&V consumption and evaluates psychological health outcomes, are required.

4.8 Conclusion

Collectively, the cross-sectional and prospective findings of this study suggest a direct, positive relationship between F&V consumption (portions and frequency) and psychological health exists. This is most consistently observed for symptoms of depression and positive psychological wellbeing. Further support was found for an indirect, cross-sectional negative relationship between savoury snacking and psychological health, via cognitive failures. Frequent sweet and savoury snacking are also cross-sectionally associated with increased stress and reduced psychological wellbeing. Moreover, frequent fruit consumption prospectively increases psychological wellbeing and reduces stress, while frequent vegetable consumption reduces symptoms of depression over time. Notably, these relationships emerged by four months, but not after one month. Overall, higher diet quality may protect memory-related cognition (reduce cognitive failures), which in turn reduces perceived stress. Therefore, dietary intake appears to be linked to specific aspects of psychological health, both directly and indirectly. Further work is required to replicate these results in order to have greater confidence in which associations are important, and to explore them from a causal approach. Future prospective, experimental and intervention studies that quantify the effects of both frequency and quantity of nutrient-rich and nutrient-poor foods on psychological health are needed.

Chapter 5 Exploring the Acute Effects of a L-methylfolate drink on Mood, Affect, Cognition, and Psychological Health in University Students; Two Pilot Experiments

The findings from Chapters 3 and 4 highlight that nutrient-rich dietary habits, such as the frequent or plentiful intake of fruit and vegetables, may be important for psychological health. However, average fruit and vegetable intake falls below the recommended levels (Statistics on Obesity, Physical Activity & Diet, England, 2020), which was also evidenced by the average intake in studies 1-3 within this thesis. Current attempts to support people to consume more vegetables, do not necessarily result in increased intake, which puts individuals at risk of key micronutrient deficiencies (Food Foundation, 2021). Several barriers to increasing vegetable intake have been reported, such as cost, access, lack of food literacy or cooking skills which make interventions difficult (Appleton et al., 2016; De Leon et al., 2020; Woodside et al., 2023). In addition, difficulties in changing existing eating behaviour are reported (Baranowski et al., 1999). However, micronutrient supplementation may promote optimal psychological outcomes through addressing nutritional inadequacies (Jacka et al., 2017; Lichtenstein & Russell, 2005). Indeed, the initial narrative review (Chapter 1) identified evidence to suggest that the optimal intake of folate is protective of mental health, after the adjustment for numerous covariates (Davison et al., 2017). Folate (also known as vitamin B9) is a micronutrient found in leafy green vegetables, such as broccoli or spinach, but can alternatively be consumed in a highly bioavailable supplement form, known as L-methylfolate. Marginal folate deficiency (also known as insufficiency or inadequate intake) has been observed in 16% of general adults and is associated with tiredness and irritability (Tardy et al., 2020). Hence, micronutrient supplementation may offer a simple solution to improving nutrient and psychological status (versus attempts to increase vegetable consumption). Thus, the following three experimental studies will focus on the acute manipulation of L-methylfolate supplementation in the form of a drink, and folate via vegetable intake, in the form of a meal, while addressing the final aim of the thesis. The final aim was to experimentally explore the effect of folate (in supplement and food form) on mood, affect, cognition, and psychological health in healthy adults in controlled laboratory settings.

5.1 Introduction

Folate (or vitamin B9) deficiency is recognised as one of the most common micronutrient deficiencies worldwide (Paiva et al., 2015). The Reference Nutrient Intake (RNI) for children and adults is at least 200mcg of folate a day, and regular intake is encouraged as the body cannot store folate (NHS, 2020). However, the National Institute of Health advise a higher RNI of 400mcg for adolescents and adults over 19 years of age (NIH, 2022). Promoting optimal folate status, i.e., a folate level associated with the lowest risk of folate-related disease, rather than merely preventing overt folate deficiency, is important across the lifespan (McNulty et al., 2019). Both folic acid (synthetic form) and dietary folate must be converted to L-methylfolate for use in the brain (Kose & Sayar, 2018). Folate is water soluble and well-tolerated (Hoepner et al., 2021), thus L-methylfolate supplementation has potential for optimising folate levels that are not met through diet alone (Commerford, 2013). Previous research has manipulated the intake of nutrients found in fruit (e.g., flavonoids, polyphenols) and observed positive effects on mood, affect, cognition, and symptoms of depression (Ali et al., 2021; Cheng et al., 2022; Khalid, 2020), but less attention has been given to the nutrients found in leafy green (cruciferous) vegetables. This is surprising because folate found in leafy green vegetables influences neurochemical levels relevant to mood, affect, cognition, and psychological health (Gilbody et al., 2007a; Morris et al., 2018). In addition, L-methylfolate has been shown to suppress inflammation and promote neural health (Jain et al., 2019; Macaluso, 2022). Consequently, there is a need for experimental work to better understand the causal relationship between folate, a bioactive micronutrient found in vegetables, and psychological health.

During adulthood, folate deficiency is associated with an increased risk of developing depression (Black, 2008). As the population continues to consume less folate from food sources such as leafy green vegetables, is it suggested that the rates and severity of depression will increase as folate consumption decreases (Barnett et al., 2017). Previous studies have utilised folic acid supplements in clinically depressed populations as an adjunctive therapy alongside antidepressants (Borges-Vieira, 2022; Lam et al., 2022). However, it is difficult to understand the direct effects separate from the interactions with medications, and any mood benefits cannot be generalised to non-clinical samples. Research shows that individuals with low serum (representing daily folate status) and red blood cell (representing longer-term) folate levels are at a higher risk of developing depression (Beydoun et al., 2010; Morris et al., 2018). Conversely, non-clinical, older adults with higher levels of folate have reported increased psychological wellbeing and positive affect (Edney et al., 2015). Furthermore, in young adults (mean age 32.5 years), those with folate intake above the recommended level (400mcg), had significantly lower scores for depressive affect but not positive affect (Yary, 2013). A systematic review of B vitamin supplementation (including B9) research in healthy adults on several aspects of psychological health identified mixed results (benefits to stress, but not symptoms of depression or anxiety) (Young et al., 2019). Furthermore, twelve-week supplementation with folic acid in healthy, non-depressed males found no benefit for positive or negative affect compared to the control group (Williams et al., 2005). A systematic review of trials examining the effects of folate supplementation for the management of depression, concluded that only the one trial involving L-methylfolate (instead of folic acid) observed benefit to depression scores, compared to the placebo control (Barnett et al., 2017). In addition, only one trial involved a healthy, non-depressed sample (Williams et al., 2005). It is conceivable that the more potent and bioavailable L-methylfolate may impact psychological health in healthy adults, but this is yet to be investigated.

Research shows that cognitive benefits are observed in healthy adults after B vitamin levels are optimised (Haskell et al., 2010; Kennedy et al., 2010). Review evidence has concluded that B group and C vitamins are the most relevant vitamins to cognitive performance (Huskisson et al., 2007). In younger adults, dietary folate intake (between 408.7 to 667.6mcg per day) has been associated with improved memory recall, speed of processing and verbal fluency in women aged 20-30 years old (Bryan et al., 2002). Furthermore, Qin and colleagues (2017) showed that young adults with high dietary intake of folate (mean intake: 384mcg/day) reported better cognitive function (verbal memory and psychomotor speed but not executive function) 20 to 25 years later in life, compared to those with the lowest folate intake (mean intake: 152mcg/day). The highest quintile of folate intake was associated with the equivalent cognitive performance gain of being 4.1 years younger in age. This suggest that improvements in cognitive performance can be observed with doses of folate aligned with RNI and at levels found in food, however, to date, no studies have tested the effect of L-methylfolate supplementation on cognitive performance.

It is unclear whether micronutrient supplementation can improve cognitive performance in younger adult populations, previous research has focussed on the improvement of mild cognitive impairment in older, and often clinical populations (Allen et al., 2013; Khalid et al., 2022). Yet, young to middle-aged adults with demanding lifestyles have been highlighted as a risk group for reduced cognitive performance, which is believed to be associated with insufficient nutrient status (Scholey, 2018). Young women have been identified as particularly at risk of nutrient inadequacy for folate (Bryan et al., 2002). Acute administration of multi-vitamins (including B9 at 200/400mcg) in healthy young adults has improved executive function and working memory during a randomised controlled trial (n= 20, mean age: 29) (Scholey et al., 2013). Moreover, functional magnetic resonance imaging (fMRI) identified increased activation in areas associated with working memory and executive attentional processing functioning, one hour following the single dose of multi-vitamins (Scholey et al., 2013). Meta-analysis evidence shows that multi-vitamin supplementation including folate improves immediate free recall but not delayed free recall, but most of the evidence is from older adult populations (Grima et al., 2012). This systematic review also highlighted that more general cognitive performance is under researched. Symptoms of folate deficiency include forgetfulness, difficulty concentrating, and fatigue (Huskisson et al., 2007), thus it is plausible that more global, memory related errors could be related to insufficient folate intake. However, it is not possible to conclude precise folate effects on cognition without specific manipulation that does not include other micronutrients.

Acute supplementation research has focussed on the multi-nutrient effects by combining vitamins and minerals to allow micronutrients to operate synergistically on physiological processes (Kennedy, 2016). This approach lacks precision for understanding the underlying biological mechanisms that may improve psychological health. Moreover, studies often involve high doses of multi-vitamins that well exceed recommended daily intakes of B vitamins, and at levels that cannot be achieved through dietary sources (Young et al., 2019). Interestingly, trials that have used multi-nutrient supplements have shown that those higher in B vitamins (including B9, folate) resulted in greater improvements in perceived stress and reduced symptomology in non-clinical samples (Long & Benton, 2013). Furthermore, a 16-week multi-nutrient liquid supplementation study in healthy older adults showed that folate explained the most variance for vitality and for general wellbeing, 8.6% and 14.2%, respectively (Krondl et al., 1999). The manipulation of folate in healthy adults could have effects on psychological wellbeing due to processes that underlie the regulation of mood (Alpert & Fava, 1997). Research suggests that of the non-pharmacological alternatives to reducing sub-clinical mood and enhancing resilience to stress, the consumption of bioactive supplements represent a promising strategy (Jackson et al., 2021). To date, studies that explore the acute effects on positive and negative mood, affect, symptoms of poor mental health or positive psychological wellbeing do not exist. Low mood and affect are interrelated and may contribute to symptoms of depression (Al Ammar et al., 2020), so reducing these could act as a resilience factor for promoting optimal psychological health.

Greater concentration and alertness have been reported after the consumption of multi-vitamin supplements, including 400mcg of folic acid compared to the control group (Kennedy et al., 2011). Improvements were observed at the end of the working day (compared with before) and across weekly assessment days which suggests an acute mode of action. A 33-day intervention of a high dose of B-complex vitamins (400mcg of folic acid included) in a cohort of psychologically healthy men showed that supplementation improved mood, ratings of mental health, cognitive performance, while also reducing subjective stress and mental tiredness (Kennedy et al., 2010). Notably, a specific reduction in negative mood symptoms was observed. Optimising mood in non-clinical populations may be a method for preventing clinical disorder. Even a single, oral dose of folate in bioavailable form (in fasted human volunteers) impacts blood folate concentrations after 30 minutes, peaking at 45 minutes (Finglas et al., 2003), so within-day acute effects may be observed through fluctuations in mood over shorter time periods. Previous studies have supplemented with 200mcg of folic acid and others with 400mcg (Kennedy et al., 2010; Long & Benton, 2013; Young et al., 2019). It is unclear whether the effects demonstrate a dose-response, linear gradient in line with the micrograms consumed because of the differences in manipulations across studies. That is, with greater folate consumption in micrograms, are greater effects on mood, affect, cognition, and psychological health observed? The psychological benefits of B vitamins after weeks or months has been documented, but the effects of L-methylfolate (at the 200mcg and 400mcg level) on mood in an acute setting with healthy, young adults is yet to be investigated.

The following two pilot experiments explore the effects of consuming folate in highly bioavailable form (L-methylfolate) at microgram levels aligned with RNI (200mcg and 400mcg) on mood, affect, cognition, and psychological health. Due to evidence of the rapid absorption of this micronutrient, the acute effects were investigated in young, healthy adults across two within-day experiments. The first pilot experiment aimed to assess proof-of-concept; whether acute supplementation of L-methylfolate (control 0mcg vs active 400mcg) has a significant effect on mood, affect, and perceptions of psychological health (mental health and wellbeing). It was hypothesised that a significant difference between the control and active condition would be observed, whereby those in the active condition would report higher positive mood or affect, lower negative mood, or affect, and better psychological health. The second pilot experiment aimed to explore if a dose-response pattern emerged for L-methylfolate micrograms levels (0mcg, 200mcg or 400mcg), and additionally, the effect of L-methylfolate on memory performance (recall memory and cognitive memory errors). It was hypothesised that participants who consumed either 200mcg or 400mcg of L-methylfolate would report higher positive mood or affect, lower negative mood, or affect, better memory performance and psychological health, in comparison to the control group (0mcg), with the largest benefits observed in the 400mcg condition.

5.2 Pilot Experiment 1: Does L-methylfolate Acutely Impact Mood, Affect and Psychological Health in Students?

5.2.1 Method

Participants

University student participants were recruited through the Research Participation Scheme (RPS) at Aston University, and they received course credits in exchange for their time. The experiment was advertised as a study of 'Hydration, Lifestyle Habits and Wellbeing;' however, participants were debriefed at the end of the testing session to explain whether they had or not consumed L-methylfolate in the water. Participants completed an initial screener questionnaire via email correspondence to ensure they met the inclusion criteria. Participants who rated their general health as poor over the last 12 months, who currently have or had diabetes, or an eating disorder and/or medically diagnosed food allergy, high blood pressure, a heart attack, or were experiencing any medical illness or taking medications were not eligible to participate. Participants were asked not to consume any caffeine 24 hours beforehand and to refrain from eating food for 2 hours prior. This is because caffeine can impair folate absorption due to a mild diuretic effect on water soluble B-vitamins (Wolde, 2014), and fasting is recommended by previous acute multi-vitamin experimental protocols (White et al., 2017). A total of 72 undergraduate students expressed an interest to take part, with 60 participants completing the pilot experiment (12 participants did not meet the screening criteria). The experiment was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample Size

Using G*Power, with number of groups set to 2, alpha set at 0.05, modelling a medium effect size (d = 0.50), and power at 80%, the minimum required sample size was 102 participants (Faul et al., 2009). However, to account for participants who might not complete the experiment in full, we aimed to recruit a minimum of 110 participants to the experiment. This sample size was not achieved due to COVID-19 restrictions that shut down the Nutrition Laboratory, and then a leave of absence was taken for maternity leave.

Design

The between-subjects factor was the L-methylfolate condition with two levels: active 400mcg or control 0mcg. There were multiple dependent variables including PANAS and DASS-21 scores (both measured once), and WHO-5 and VAS scores (both measured at 15-minute intervals, and thus, a within-subjects factor of 'time' applies to these). Participants completed one testing session for the total duration of approximately 50-55 minutes, and this took place in the Aston University Psychology Laboratory. Participants were randomly assigned to consume either L-methylfolate enriched water (at the 400mcg level), or no L-methylfolate, plain water (the control).

Measures

Screener questions and eligibility check: Screener questions were emailed to participants once they expressed an interest in taking part through the RPS. These were simple questions to assess the inclusion criteria of reporting good or better general health, no medical illness, not having diabetes, a heart condition, an eating disorder, any food allergies, and not currently taking any medications. Additionally, an eligibility check was administered upon arrival at the laboratory to ensure participants had not consumed caffeine 24 hours before, or food in the 2 hours before the experiment.

Demographic and lifestyle questionnaire: This included questions on sex, age, ethnicity, household income, alcohol intake, smoking status and total cigarettes smoked to date, and a food recall for the current day (to ensure participants were fasted). These questions were used to characterise the sample and assess potential covariates.

The Short-form Food Frequency Questionnaire (SF-FFQ): The SF-FFQ was used to examine the consumption (frequency and portions) of foods in a “typical” week, over the past month using an 8-point Likert scale (rarely or never to 5+ a day) (Cleghorn & Cade, 2016). The food groups investigated in this experiment were: (1) fruit consumption, which included fresh or tinned; (2) vegetable consumption, which included fresh, tinned, or frozen, but not potatoes; (3) green, leafy vegetable consumption, for example spinach, broccoli, and kale; (4) sweet snacking, which included biscuits, cakes, chocolate, and sweets, and; (5) savoury snacking, which included crisps or savoury snacks. This measure of habitual food consumption has been used throughout the thesis and therefore previously discussed in more detail.

The Three-Factor Eating Questionnaire (TFEQ-21): The 21-item TFEQ (Cappelleri et al., 2009) assessed participants’ eating style using items such as, ‘I don’t eat some foods because they make me fat’ and ‘I start to eat when I feel anxious.’ Participants rated each item on a 4-point Likert scale, for example from ‘definitely false’ to ‘definitely true’ and the last question requires participants to rate their restraint in eating on a scale from 1 to 8. The TFEQ is a reliable measure of uncontrolled eating, cognitive restraint, and emotional eating (Cappelleri et al., 2009).

The World Health Organisation- 5 Wellbeing Index (WHO-5): The WHO-5 (Topp, et al., 2015) was used to assess perceptions of positive psychological wellbeing. The WHO-

5 is a short, 5-item self-administered questionnaire that assesses the current wellbeing of participants. The WHO-5 was deemed more appropriate for repeated measurements as it is quicker to complete than the WEMWBS, which is better suited to evaluating changes related to interventions at a group level (Maheswaran et al., 2012). The WHO-5 has high sensitivity and specificity (Topp et al., 2015). Each item is positively phrased for example, 'I have felt cheerful and in good spirits' and asks participants to consider each over the last 14 days. The response ratings range from 0 (none of the time) to 5 (all of the time), therefore the minimum and maximum score is between 0 to 25. The WHO-5 has good validity for measuring general positive wellbeing in the young adult population (Hall et al., 2011).

Visual Analogue Scales (VAS): Eleven VAS (Crichton, 2001) were used to measure mood throughout the experiment, these included how alert, drowsy, anxious, happy, sad, withdrawn, stressed, content, inspired, calm, and irritable participants felt right in the current moment. In addition, three appetitive moods including: hunger, thirst, and desire to eat were assessed, but only analysed at baseline to determine if any differences between the active and control conditions exist prior to the manipulation. For instance, large differences in how thirsty participants are could play a role in the effects on mood ratings after consuming the drink. Participants rated the degree to which they were experiencing each mood on a scale from 0 (not at all) to 100 (very much) in the present moment.

The Positive and Negative Affect Schedule (PANAS): The PANAS (Watson et al., 1988) was used to assess positive and negative affect (10 items for each) as an additional measure to the VAS, to allow for comparisons between current mood (VAS) and affect ratings from the past week. In other words, in-moment mood and recent affect were assessed alongside each other. The PANAS is a 20-item self-report measure that asks participants to rate the degree to which they have felt for example, 'Interested' and 'Irritable', in the past week on a 5-point Likert scale from 'very slightly/not at all' to 'extremely'. Scores are computed to produce an overall positive and negative affect score that ranges from 10-50. Higher scores indicate higher positive or negative affect. Research has shown that the PANAS is a valid and reliable measure of positive and negative affect in a non-clinical adult UK population (Crawford & Henry, 2004).

Depression, Anxiety and Stress Scale -21 Items (DASS-21): The Depression Anxiety and Stress Scale (Lovibond & Lovibond, 1995) was used to measure symptoms of depression, anxiety, and stress over the past week. The validity and reliability of the DASS-21 among student samples is well established (McDowell, 2006), hence it was deemed a more appropriate measure than the HADs for use within the current student samples. Furthermore, the DASS-21 includes the additional dimension of stress, not covered by the HADS, and has shown superior internal consistency over the HADS (Sukantarat et al., 2007). Each of the three subscales (depression, anxiety, stress) were computed to provide a total score for each aspect of psychological health. Participants rated items such as, 'I found it hard to wind down' and 'I felt scared without any good reason' on a scale from 0 (did not apply to me at all) to 3 (applied to me very much or most of the time). The DASS-21 was found to have Chronbach's alpha scores of 0.81 (depression), 0.89 (anxiety) and 0.78 (stress) for each subscale and to have good concurrent and convergent validity (Gloster et al., 2008).

The International Physical Activity Questionnaire Short form (IPAQ): To characterise the sample and examine any potential baseline differences between groups for other key lifestyle behaviours (i.e., exercise and sleep), the IPAQ (Craig et al., 2003), a 7-item questionnaire was used to measure the participants' self-reported physical activity levels and sedentary behaviour. The questions require participants to report how many days per week, hours per day or minutes per day they spent doing various physical activities (during the past week). Dinger et al. (2006) found that time spent in vigorous and moderate physical activity according to the IPAQ significantly correlates with accelerometer measures of steps per day for university students.

The Pittsburgh Sleep Quality Index (PSQI): The PSQI (Buysse et al.,1989) is a 10-item questionnaire used to assess participants' sleep by measuring their sleep latency, duration, efficiency, sleep disturbances, use of sleep medication and daytime dysfunction. The items ask participants to report sleep experiences from the past month. The PSQI is a valid measure of sleep in the university student population (Dietch et al., 2016).

Hydration questions: These were inserted to maintain the cover story for the experiment; however, the answers were not analysed. The hydration questions included: 'What is the recommended amount of water you should drink per day?' and 'What are some of the symptoms of dehydration?'

Height and Weight: Within the laboratory, a stadiometer and scales were used to measure height (cm) and weight (kg). These were then used to calculate a BMI score for each participant.

L-methylfolate manipulation

The highly bioavailable, liquid form of folate known as L-methylfolate was used to manipulate folate consumption, and manufactured by Biocare; Nutrisorb® Methylfolate 15ml (biocare.co.uk). When consumed orally, this form of folate crosses the blood-brain barrier without the need for conversion (unlike folic acid) (Roman & Bembry, 2011). L-methylfolate is considered to impact plasma folate concentrations after 30 minutes, peaking at 45 minutes (Finglas et al., 2003). The recommended adult daily intake of folate is 400 mcg as a dietary supplement, and four drops of liquid L-methylfolate are equivalent to this (Office of Dietary Supplements, 2019). The experimenter prepared the drink before the participant arrived; this comprised 125ml of water with either 400mcg (4 drops) of L-methylfolate added or left plain (control). Previous pilot testing indicated that the L-methylfolate drink was odourless, colourless, and tasteless in the water, ensuring that the participants were blind to which group they were in.

Procedure

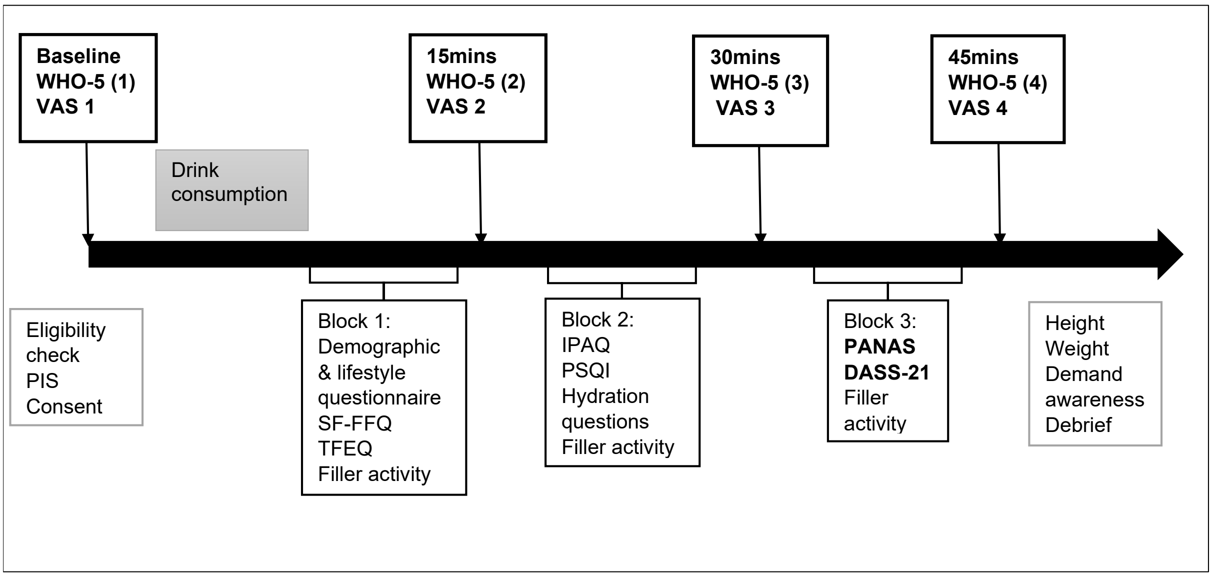
Participants who expressed a willingness to take part through the University RPS were sent initial screener information and made aware of the inclusion criteria. Eligible participants were told not consume caffeine 24 hours prior to the experiment and to consume food no later than 2 hours before attending the laboratory. Upon arrival at the Psychology department laboratory, a final eligibility check was completed, and participants were sat at a computer to record their responses within Qualtrics, the web-based software for hosting surveys.

Firstly, participants read the information sheet (which was also emailed to them at least 24 hours before), as per the research advert, this posed the experiment as an exploration of the effects of hydration on wellbeing. Following this, participants provided informed consent and completed the baseline wellbeing (WHO-5) and mood (VAS) ratings. Participants then consumed a drink of 125 ml of water (with or without) four drops of liquid L-methylfolate added. Participants were randomly allocated to either the active or control condition using the Excel random selection formula within a spreadsheet.

After the drink was consumed, participants were asked to complete a series of online questionnaires. The first block of questionnaires involved completion of a demographic and lifestyle questionnaire, the SF-FFQ and the TFEQ, with the filler activity. The second block involved completion of the IPAQ, the PSQI, hydration questions and the filler activity. The third block included the PANAS, DASS-21, and the filler activity. Between these three blocks the WHO-5 and VAS were completed at 15 and 30 minutes, which are the key measures of interest completed at 15-minute intervals throughout the experiment. After the third block, the final WHO-5 and VAS were completed at 45 minutes, followed by the final measures of the participant’s height and weight using the stadiometer and scales. Participants were then debriefed, the aims of the experiment were clarified, and for those in the active condition, it was explained to participants that they had consumed their daily intake of folate in liquid form via the drink of water.

In total, the WHO-5 and VAS were completed four times over the course of the experiment. Participant height and weight were measured after the final WHO-5 and VAS to avoid any effects on participant mood. The total duration of the experiment was approximately 50-55 minutes. The timing of measures was recorded throughout the experiment on a mobile phone's stopwatch to maintain the procedural order (see Figure 5.1). When required, the filler activity of asking participants to read neutral magazines (furniture or stationery related) was implemented to ensure consistent timings across experiments. Participants were provided with a selection of magazines to read, these were furniture (Ikea, Muiji) and stationary (Ryman's, Muiji) related.

Figure 5.1 Pilot experiment 1 procedural order.



Note: Measures in bold text represent the key measures. Measure abbreviations: Participant Information Sheet (PIS), World Health Organisation-5 item wellbeing scale (WHO-5), Visual analogue scales (VAS), Short-form food frequency questionnaire (SF-FFQ), Three-factor eating questionnaire (TFEQ), International physical activity questionnaire (IPAQ), Pittsburgh sleep quality index (PSQI), Positive and negative affect schedule (PANAS), Depression anxiety stress scale (DASS-21).

Statistical analyses

***Participant characteristics and baseline scores*:** were assessed using t-tests to determine whether significant differences between the two conditions existed at baseline. Descriptive data were calculated as means with standard deviation, or as frequencies (%) for categorical variables.

Covariates: Pearson's correlations for theoretical covariates revealed that household income was the only covariate that significantly, negatively correlated with two aspects of psychological health (DASS-21 anxiety and stress scores, *ps* <.05), this was therefore included in the following ANCOVA analyses. Furthermore, baseline thirst rating was included as a covariate because this was found to be significant between conditions (lower mean thirst scores in the active compared to the control condition). In addition, as discussed during the method, this experiment was posed as an exploration of the effects of hydration on wellbeing, hence inclusion of baseline thirst rating was deemed appropriate as an additional covariate. None of the other theoretical measures correlated with at least two of the outcomes, thus were not included as covariates.

Main analyses: Due to the wellbeing (WHO-5) and mood (VAS) measures being completed at multiple time points, the data were converted into an Area Under the Curve (AUC) score to allow for total fluctuations as a function of time to be studied. The AUC score was computed using the trapezoidal rule method (Le Floch et al., 1990) which calculates the fluctuations in mood from baseline through to the last measure taken at 45 minutes. A spreadsheet in Excel was used to apply the integral formula and calculate each AUC score. The analyses involved independent samples t-tests for the AUC scores, and one-way ANCOVA analyses with covariates for the psychological health outcomes, to identify whether a significant difference between the active (400mcg) and control (0mcg) conditions were observed for wellbeing scores, mood ratings, affect and perceived psychological health (depression, anxiety, and stress).

5.2.2 Results

Participant characteristics and baseline scores

Sixty participants completed the experiment with an overall mean age of 20.33 (SD = 1.57), 81.7% were female (n = 49) and 18.3% (n = 11) were male. The participants' ethnic background was as follows: 52% Asian, 27% White, 14% Black, 2% Chinese, and 5% were mixed ethnicities. Participant's average BMI was within a healthy range (mean = 23.59, SD = 7.59). The majority of participants' household income for the past 12 months (before taxes and from all sources) was between £10,000 and £49,999. Eight percent were smokers and 44% drank alcohol. Seventy-five percent of participants averaged 7+ hours of sleep per night and 4% of participants engaged in vigorous (e.g., heavy lifting or aerobics) physical activity once a day. For the overall sample, the DASS-21 scores indicated a non-clinical sample (DASS-21 mean: depression = 12.4 (SD = 4.6), anxiety = 12.1 (SD = 3.4), stress = 14.2 (SD = 3.5).

Demographic information, lifestyle behaviour information, and baseline mood ratings by condition are presented in Table 5.1. At baseline, there were no significant differences between conditions for demographic, lifestyle, and appetite and mood ratings, except for age (younger participants in the active condition) and thirst rating (lower thirst rating in the active condition). However, both conditions included participants aged between 18 and 23 years old. There were no significant differences between conditions for BMI, eating habits (daily portions) or eating style (shown in Table 5.2). For food frequency (SF-FFQ), on average, participants consumed fruit 2-3 times a week, and vegetables 4-6 times a week, savoury snacks 2-3 times a week, and sweet snacks 4-6 times a week. Overall, participants consumed on average 1.74 portions (SD = 1.25) of fruit a day, 2.15 portions (SD = 1.30) of vegetables a day, and 1.15 portions (SD = .79) of green, leafy vegetables (spinach, broccoli etc.). For further information regarding participants' eating habits, see Table 5.2.

Table 5.1 Baseline demographic, lifestyle behaviours, and mood ratings by condition.

N, mean (SD or %)	**Control n = 30**	**Active n = 30**	***p***
Demographic			
Sex (%)			
Female	25 (83)	24 (80)	
Male	5 (17)	6 (20)	
Mean age (SD)	20.9 (1.8)	19.8 (1.2)	.008
Household income	20-49K	20-49K	.569
Lifestyle behaviours			
Physical activity total minutes last week (SD)	134.5 (190.4)	174.7 (216.1)	.448
Sleep duration (SD)	7.5 (1.3)	7.3 (1.2)	.538
Alcohol intake (units per week)	<14 units	<14 units	.855
Smoking status (cigarettes total)	Never smoked > 100 cigarettes	Never smoked > 100 cigarettes	.274
Baseline Mood and Appetite Ratings (VAS)			
Calm	53.6 (30.3)	66.2 (26.8)	.095
Hungry	52.0 (34.1)	52.3 (33.0)	.976
Thirsty	40.2 (27.7)	22.0 (23.7)	.008
Desire to Eat	53.3 (35.7)	53.13 (32.4)	.985

Note: n = Number. SD = Standard Deviation

Table 5.2 Participants' Body Mass Index (BMI), eating habits and style by condition.

Mean (SD)	**Control n = 30**	**Active n = 30**	***p***
BMI	25.0 (10.8)	22.5 (3.9)	.276
Fruit intake (portions)	1.8 (1.2)	1.7 (1.3)	.748
Vegetable intake (portions)	2.2 (1.1)	2.1 (1.4)	.786
Leafy green vegetable intake (portions)	1.2 (.5)	1.1 (.9)	.665
Sweet snacking (portions)	1.0 (.7)	1.6 (1.2)	.056

Savoury snacking (portions)	1.0 (.9)	1.1 (.8)	.447
Uncontrolled eating	2.2 (0.4)	2.4 (0.4)	.061
Cognitive restraint	2.2 (0.7)	2.2 (0.7)	.924
Emotional eating	2.2 (0.7)	2.4 (0.7)	.352

Note: n = Number. SD = Standard Deviation

Main analyses

Area Under the Curve wellbeing and mood scores: Independent samples t-tests were conducted to examine the differences between conditions for wellbeing (WHO-5) and mood ratings (VAS) scores. These revealed several significant differences (all *ps* <.05 - please see Table 5.3, below). For negative moods, this included lower ratings of drowsiness, anxiousness, sadness, feeling withdrawn, stressed, and irritable, in the active condition compared to the control. For positive moods, this included higher ratings of feeling alert and content in the active condition compared to the control. There were no further significant differences in mood ratings and no significant difference between conditions for WHO-5 wellbeing scores.

Table 5.3 Independent t-test results for wellbeing (WHO-5) and mood ratings (VAS 1-4, baseline to 45 minutes) Area Under the Curve scores by condition.

	Control	**Active**	
	Mean (SD)	**Mean (SD)**	***p***
Wellbeing (WHO-5)	1075 (213)	1102 (165)	.596
VAS			
Alert	2503 (1099)	3016 (661)	.033*
Drowsy	1193 (1146)	519 (501)	.005**
Anxious	1127 (1100)	300 (293)	<.001***
Happy	2126 (1003)	2545 (772)	.75
Sad	1150 (1188)	375 (410)	.002**
Withdrawn	1194 (1116)	265 (287)	<.001***
Stressed	1644 (1058)	850 (620)	<.001***
Content	1851 (1094)	2399 (1011)	.049*
Inspired	1232 (957)	1592 (1010)	.163
Calm	2373 (1016)	2712 (920)	.180
Irritable	1330 (1132)	387 (499)	<.001***

Note: $^{*}p$ <.05, $^{**}p$ < .01, $^{***}p$ <.001.

Psychological health and affect ratings: One-way ANCOVA analyses were conducted including baseline thirst rating and household income as covariates, to examine the differences between the control and active condition on subjective psychological health ratings. For depression scores, there was no significant main effect for condition (F(1,56) =

3.074, p = .085, η^2 = .052, observed power = .407), and this was also the case for anxiety scores (F(1,56) = .318, p = .575, η^2 = .006, observed power = .086.), and for stress scores, (F(1,56) = 1.749, p = .191, η^2 = .030, observed power = .255). However, for positive affect (PANAS), there was a significant main effect of condition (F(1,56) = 17.452, p <.001, η^2 = .238, observed power = .984), whereby those in the L-methylfolate condition scored higher for positive affect compared to those in the control condition, and for negative affect, there was a significant main effect of condition (F(1,56) = 8.194, p =.006, η^2 = .128, observed power = .803), whereby those in the L-methylfolate condition scored lower for negative affect compared to those in the control condition (see Table 5.4 for the means and SD).

Table 5.4 Means and standard deviation (SD) of affect and psychological health ratings by condition (one-way ANCOVA).

	Control	**Active**
Measure	**Mean (SD)**	**Mean (SD)**
Depression (DASS-21)	13.13 (4.90)	11.57 (4.10)
Anxiety (DASS-21)	12.10 (3.74)	12.20 (3.18)
Stress (DASS-21)	14.57 (3.94)	13.90 (3.04)
Positive Affect (PANAS)	26.20 (7.20)	32.13 (5.47)***
Negative Affect (PANAS)	22.43 (7.0)	19.23 (5.23)**

Note: **p < .01, ***p <.001. Covariates = baseline thirst rating and household income.

5.3 Pilot Experiment 2: Is there an Acute, Dose-Response Effect of L-methylfolate on Mood, Affect, Cognition, and Psychological Health in Students?

5.3.1 Method

Participants

University student participants were recruited through the Research Participation Scheme (RPS) at Aston University, and they received course credits in exchange for their time. This pilot experiment involved a separate university student sample to the preceding study. Again, the experiment was advertised as a study of 'Hydration, Lifestyle Habits, and Wellbeing;' however, participants were debriefed at the end of the testing session to explain whether they had or not consumed L-methylfolate in the water. The same exclusion and eligibility criteria as pilot experiment 1 was used. A total of 83 undergraduate students expressed an interest to take part, with 65 participants completing the pilot experiment (15 participants did not meet the screened criteria, 3 consumed caffeine before participating, thus were excluded during the eligibility check upon arrival). The experiment was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample size

Using G*Power, with number of groups set to 3, alpha set at 0.05, modelling a medium effect size (*f* = 0.25), and power at 80%, the minimum required sample size was 159 participants (Faul et al., 2009). However, to account for participants who might not complete the experiment in full, we aimed to enrol a minimum of 165 participants to the study. This sample size was not achieved due to COVID-19 restrictions that shut down the Nutrition Laboratory, and then a leave of absence was taken for maternity leave.

Design

The between-subjects factor was the L-methylfolate condition with three levels: 0mcg, 200mcg or 400mcg. There were multiple dependent variables including WHO-5, VAS, PANAS (each measured at 15-minute intervals), the WEMWBS and the DASS-21 scores (completed once), and thus, a within-subjects factor of 'time' applies to these. Additional cognitive dependent variables were the number of words recalled during the recall task and the CFQ scores. Participants completed one testing session for the duration of approximately 65-70 minutes, and this took place in the Aston University Psychology Laboratory. Participants were randomly assigned to consume either: L-methylfolate enriched water (at the 200mcg level), L-methylfolate enriched water (at the 400mcg level), or no L-methylfolate, plain water (the control).

Measures

The measures used during the first pilot experiment were used again during this second pilot experiment, with the following additional measures. Cognitive measures were added into the second pilot due to the longer duration; this included a word list delayed-recall task and the cognitive failures questionnaire to explore memory-related outcomes.

General Health Rating: General health was assessed using a single item with a five-point Likert scale (ranging from poor to excellent) asking participants to rate: "Over the last 12 months, would you say that on the whole, your health has been…" For this pilot experiment, general health rating was not only assessed as part of the screening process, but also explicitly for potential inclusion as a covariate.

The Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS): The 14-item WEMWBS scale (Stewart-Brown & Janmohamed, 2008) was used to assess positive psychological wellbeing alongside the WHO-5 (Topp et al., 2015), this was added in to compare against the WHO-5 due to the null findings in the previous pilot study. As previously mentioned, it is inappropriate for repeated use, slower to complete and less sensitive to change than the WHO-5. All items are positively worded and cover both feelings ('I've been feeling relaxed') and thoughts (I've been thinking clearly') related to wellbeing. Participants self-report the extent to which they have experienced each item over the previous two weeks, using the 5-point Likert scale ranging from 'none of the time' to 'all of the time'. The WEMWBS is significantly correlated with other mental health and wellbeing scales and has been shown to have a Cronbach's alpha score of 0.89 in the student population (Tennant et al., 2007).

Word list delayed-recall task: The delayed-recall task was used to test the participants' cognitive ability of memory retrieval. Participants were presented with a list of 20 nouns at the start of the experiment for a total duration of 1 minute. Then, at the 55 minutes point of the experiment, participants were asked to recall as many words as possible within a total duration of three minutes. The number of nouns correctly recalled was the outcome measure. This word list recall task was adapted from Stollery & Christian, (2013). The nouns were randomly selected from the MRC psycholinguistic database: Machine Usable Dictionary (Wilson, 1988). Each noun was six letters long and a variety of low frequency of use (i.e., cymbal), high frequency of us (i.e., butter), low imageability (i.e., poetry) and high imageability (i.e., tongue) nouns were used, see Appendix A for the word list.

The Cognitive Failures Questionnaire (CFQ): The 25-item CFQ (Broadbent et al., 1982) was used to assess participants' frequency of everyday cognitive lapses in perception and memory in the past six months. Participants reported how often they made mistakes such as, 'do you forget why you went from one part of the house to the other?' on a 5-point Likert scale from 0 (never) to 4 (very often). One item was adapted: "Do you leave important letters unanswered for days?" whereby "letters" was changed to "emails." A higher total score indicates an increased tendency to experience cognitive failures. The CFQ has good internal consistency and test-retest reliability (0.82 over 2 months) (vom Hofe et al., 1998).

L-methylfolate manipulation

The dose in micrograms of L-methylfolate was manipulated to extend the previous pilot experiment. The experimenter prepared the drink before the participant arrived. This comprised 125ml of water with either 200mcg (2 drops) or 400mcg (4 drops) of L-methylfolate added, or left plain (control). L-methylfolate is considered to impact plasma folate concentrations after 30 minutes, peaking at 45 minutes (Finglas et al., 2003).

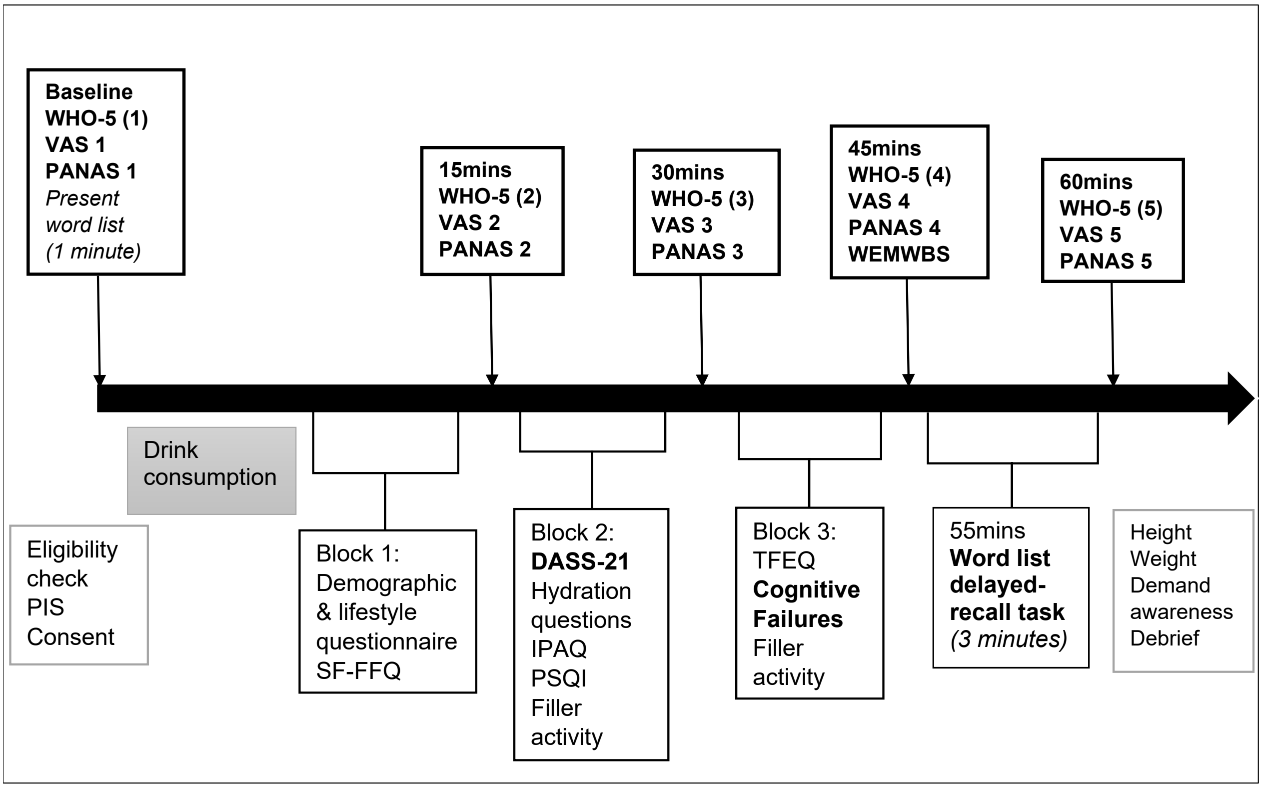
Procedure

Participants who expressed a willingness to take part through the University RPS were sent initial screener information and made aware of the inclusion criteria. Eligible participants were told not consume caffeine 24 hours prior to the experiment and to consume food no later than 2 hours before attending the laboratory. Upon arrival at the Psychology department laboratory, a final eligibility check was completed, and participants were sat at a computer to record their responses within Qualtrics, the web-based software for hosting surveys. Firstly, participants read the information sheet (which was also emailed to them at least 24 hours before), as per the research advert, this posed the experiment as an exploration of the effects of hydration on wellbeing. Following this, participants provided informed consent and completed baseline wellbeing (WHO-5), mood (VAS) and affect (PANAS) ratings. Participants were then presented with a word list of 20 nouns and given 1 minute (timed on a phone stopwatch) to memorise the words. Participants then consumed a drink according to their random allocation, using the Excel random selection formula within a spreadsheet.

After the drink was consumed, participants were asked to complete a series of online questionnaires. The first block of questionnaires involved completion of a demographic and lifestyle questionnaire, then the SF-FFQ. The second block involved completion of the DASS-21, the hydration questions, IPAQ, PSQI, and the filler activity. The third block included the TFEQ, CFQ and the filler activity. Between these three blocks the WHO-5, VAS and PANAS were completed at 15 and 30 minutes, which are the key measures of interest completed at 15-minute intervals throughout the experiment. After the third block, participants completed the fourth WHO-5, VAS, PANAS and second WEMWBS at 45 minutes, then at 55 minutes the word list recall task. This involved recall of as many words as they could in 3 minutes from the list of 20 nouns presented to them at the beginning of the experiment (see Appendix A for the word list). This was followed by the final WHO-5, VAS and PANAS, which were completed at 60 minutes. Finally, the participant's height and weight were measured using the stadiometer and scales, participants were debriefed, and the aims of the experiment were clarified. For those in the L-methylfolate conditions, it was explained to participants that they had consumed their daily intake of folate in liquid form via the drink of water.

The total duration of the pilot experiment was approximately 65-70 minutes. In total, the WHO-5, VAS and PANAS were completed five times over the course of the experiment. The timing of measures was recorded throughout the experiment on a mobile phone's stopwatch to maintain the procedural order (see Figure 5.2). When required, the filler activity of asking participants to read neutral magazines (furniture or stationery related) was implemented to ensure consistent timings across experiments.

Figure 5.2 Pilot experiment 2 procedural order.



Note: Measures in bold text represent the key measures. Additional measure abbreviations: Warwick-Edinburgh mental wellbeing scale (WEMWBS). Measure abbreviations: Participant Information Sheet (PIS), World Health Organisation-5 item wellbeing scale (WHO-5), Visual analogue scales (VAS), Short-form food frequency questionnaire (SF-FFQ), Three-factor eating questionnaire (TFEQ), International physical activity questionnaire (IPAQ), Pittsburgh sleep quality index (PSQI), Positive and negative affect schedule (PANAS), Depression anxiety stress scale (DASS-21).

Statistical analyses

Participant characteristics and baseline scores: were assessed using one-way ANOVAs to determine whether significant differences between the three conditions existed at baseline. Descriptive data were calculated as means with standard deviation, or as frequencies (%) for categorical variables.

Covariates: Pearson's correlations for theoretical covariates revealed that general health rating was significantly correlated with five aspects of psychological health, this included negative affect PANAS score, WEMWBS wellbeing score and all DASS-21 (depression, anxiety, stress) psychological health scores (*ps* <.05), thus was included in the following ANCOVA analyses. Furthermore, baseline thirst rating was included as a covariate because this experiment was posed as an exploration of the effects of hydration on wellbeing, and upon evaluation this was significantly, positively associated with negative affect scores (completed at 45 minutes p = .004, and 60 mins p = .023). Therefore, inclusion of baseline thirst rating was deemed appropriate as an additional covariate, and this was also included in the first pilot experiment. Emotional eating scores (TFEQ) were not significantly correlated with any of the cognition or psychological health outcomes (*ps* >.05). So, even though there was a significant difference between conditions found at baseline, this was not included as a covariate. None of the other theoretical measures correlated with at least two of the outcomes (age negatively with only WEMWBS wellbeing score; sleep duration positively with only WEMWBS wellbeing score), thus were not included as covariates.

Main analyses: Due to the wellbeing (WHO-5), mood (VAS) and affect (PANAS) measures being completed at multiple time points, the data were converted into an Area Under the Curve (AUC) score to allow for total fluctuations as a function of time to be studied. The AUC score was computed using the trapezoidal rule method (Le Floch et al., 1990) which considered the fluctuations in scores from baseline through to the last measure taken at 60 minutes. A spreadsheet in Excel was used to apply the integral formula and calculate each AUC score. The analyses involved one-way ANOVA for the AUC scores, and one-way ANCOVA analyses with covariates for the cognitive and psychological health outcomes, to identify whether a significant difference between the two active conditions (400mcg, 200mcg) and control condition (0mcg) were observed for wellbeing scores (WEMWBS), mood ratings, affect and perceived psychological health (depression, anxiety, and stress). The condition means will be used to evaluate any potential dose-dependent relationships.

5.3.2 Results

Participant characteristics and baseline scores

Sixty-five participants completed the experiment with an overall mean age of 19.4 (SD = 2.58), 77% were female (n = 50) and 23% (n = 15) were male. The participants' ethnic background was as follows: 57% Asian, 18% White, 14% Black, and 11% were mixed ethnicities. Participant's average BMI was within a healthy range (mean = 24.5, SD = 4.23). The majority of participants' household income for the past 12 months (before taxes and from all sources) was between £10,000 and £49,999. Five percent were smokers and 24% drank alcohol. Sixty-three percent of participants averaged 7+ hours of sleep per night, and 12% of participants engaged in vigorous (e.g., heavy lifting or aerobics) physical activity once a day. For the overall sample, the DASS-21 scores indicated a non-clinical sample (DASS-21 mean: depression = 11.4 (SD = 3.9), anxiety = 11.0 (SD = 3.3), stress = 12.0 (SD = 3.6).

Further descriptive data by condition is presented in Tables 5.5 and 5.6. At baseline, there were no significant differences between condition for demographic, lifestyle, and mood ratings, nor were there any significant differences between condition for BMI, eating habits (daily portions) or eating style, except for emotional eating which was slightly higher in the 200mcg condition. For food frequency (SF-FFQ), on average, participants consumed fruit and vegetables 2-3 times a week, and sweet and savoury snacks 2-3 times a week. Overall, participants consumed on average 1.80 portions (SD = 1.0) of fruit a day, 1.82 portions (SD = .93) of vegetables a day, and 1.06 portions (SD = .95) of leafy green vegetables (spinach, broccoli etc.). For further information regarding participants' eating habits, see Table 5.6.

Table 5.5 Baseline demographic, lifestyle behaviours, and mood ratings by condition.

N, mean (SD or %)	**Control n = 25**	**200mcg n = 22**	**400mcg n = 18**	***p***
Demographic				
Sex (%)				
Female	18 (72)	18 (82)	14 (78)	
Male	7 (28)	4 (18)	4 (22)	
Mean age (SD)	19.1 (1.0)	20.1 (4.3)	18.9 (0.8)	.310
Household income	20-49K	20-49K	20-49K	.912
Lifestyle behaviours				
Physical activity total minutes last week (SD)	161.1 (124.2)	206.6 (157.2)	247.1 (140.3)	.145
Sleep duration (SD)	6.9 (1.0)	6.8 (1.3)	7.0 (1.6)	.786
Alcohol intake (units per week)	<14 units	<14 units	<14 units	.853
Smoking status (cigarettes total)	<100	<100	<100	.676
General health rating	Good	Good	Good	.752
Baseline Mood Ratings (VAS)				
Calm	62.6 (20.3)	63.5 (25.9)	70.7 (22.4)	.476
Hungry	53.8 (30.6)	59.8 (24.8)	54.9 (30.5)	.761
Thirsty	64.7 (27.3)	60.6 (25.7)	56.3 (33.8)	.640
Desire to Eat	54.2 (30.9)	65.3 (23.1)	61.9 (29.9)	.389

Note: n = Number, SD = Standard Deviation

Table 5.6 Participants' Body Mass Index (BMI), eating habits and style by condition.

Mean (SD)	**Control n = 25**	**200mcg n = 22**	**400mcg n = 18**	***p***
BMI	24.6 (3.5)	23.8 (3.6)	25.0 (5.8)	.659
Fruit intake (portions)	1.8 (1.0)	2.0 (1.1)	1.5 (0.9)	.234
Vegetable intake (portions)	1.7 (0.9)	1.9 (0.8)	1.8 (1.1)	.788

Leafy green vegetable intake (portions)	1.3 (1.1)	0.9 (0.8)	0.9 (0.8)	.217
Sweet snacking (portions)	1.6 (1.1)	1.4 (1.0)	1.6 (1.3)	.720
Savoury snacking (portions)	1.2 (1.0)	1.4 (1.2)	1.4 (0.9)	.794
Uncontrolled eating	2.8 (0.5)	3.1 (0.5)	2.9 (0.7)	.328
Cognitive restraint	2.5 (0.6)	2.5 (0.5)	2.4 (0.6)	.739
Emotional eating	2.7 (0.4)	3.1 (0.5)	2.9 (0.6)	.029

Note: n = Number, SD = Standard Deviation

Main analyses

Area Under the Curve wellbeing, affect and mood scores: One-way ANOVA analyses were conducted to examine the differences between condition for wellbeing (WHO-5), positive and negative affect (PANAS), and mood ratings (VAS) Area Under the Curve scores. These revealed no significant differences (all *ps* >.05), please see Table 5.7 for the statistical values, presented below.

Table 5.7 One-way ANOVA results for wellbeing (WHO-5), affect (PANAS) and mood ratings (VAS 1-5, baseline to 60 minutes) Area Under the Curve scores by condition.

	Condition			
	Control	**200mcg**	**400mcg**	***p***
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
Wellbeing (WHO-5)	939 (258)	888 (297)	1049 (213)	.152
Positive affect (PANAS)	1746 (375)	1667 (462)	1834 (399)	.451
Negative affect (PANAS)	1026 (351)	1058 (388)	970 (345)	.744
VAS				
Alert	3334 (837)	3168 (1223)	3594 (346)	.520
Drowsy	1549 (1267)	1821 (1263)	1254 (1295)	.380
Anxious	713 (712)	816 (984)	453 (633)	.349
Happy	2791 (240)	3150 (264)	3577 (275)	.116
Sad	924 (852)	815 (966)	746 (1174)	.837
Withdrawn	1464 (1302)	935 (1013)	780 (955)	.109
Stress	1164 (1126)	1594 (1437)	1047 (1112)	.327
Content	2782 (935)	2978 (1406)	3528 (1388)	.158
Inspired	2107 (1145)	2590 (1597)	2969 (1413)	.133
Calm	3727 (940)	3621 (1139)	3958 (1130)	.602
Irritable	1463 (1230)	1344 (1289)	914 (976)	.313

Cognition and subjective psychological health ratings: One-way ANCOVA analyses were conducted including general health and baseline thirst rating as covariates, to examine the differences between conditions for cognition and psychological health scores.

These revealed no significant main effects of condition on cognition (cognitive failures or word list recall task); for cognitive failures scores (F(2,59) = .409, p = .666, η^2 = .014, observed power = .113.); nor delayed-word recall scores (F(2,59) = 1.319, p = .275, η^2 = .043, observed power = .274.) No significant main effects of condition on depression (F(2,59) = .249, p = .781, η^2 = .008, observed power = .087), anxiety (F(2,59) = .937, p = .397, η^2 = .031, observed power = .205), or stress (F(2,59) = .201, p = .818, η^2 = .007, observed power = .080) DASS-21 subscale scores were found. For wellbeing (WEMWBS), there was no significant main effect of condition (F(2,59) = 2.952, p = .060, η^2 = .091, observed power = .554). Please see Table 5.8 for the means and SD, presented below. Furthermore, positive, and negative affect PANAS scores were explored at the 45-minute timepoint (in addition to the AUC scores, in Table 5.7 above), and no significant main effects were observed (*ps* >.05).

Table 5.8 Means and standard deviation (SD) of cognition and psychological health ratings by condition (one-way ANCOVA).

	Condition		
	0mcg	**200mcg**	**400mcg**
Measure	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Cognitive Failures	44.44 (15.54)	42.50 (12.52)	39.44 (17.24)
Word Recall	3.96 (1.90)	5.18 (3.85)	5.11 (2.74)
Depression (DASS-21)	11.80 (3.46)	11.55 (4.35)	10.56 (3.88)
Anxiety (DASS-21)	11.92 (3.19)	10.77 (3.54)	10.33 (3.03)
Stress (DASS-21)	12.28 (3.80)	11.68 (3.33)	11.78 (3.87)
Wellbeing (WEMWBS)	43.40 (8.51)	44.09 (9.48)	50.83 (10.74)

Note: Covariates = general health rating and baseline thirst rating.

5.4 Discussion

Findings from the first pilot experiment provide initial proof-of-concept that L-methylfolate supplementation (at the 400mcg level) is beneficial for reducing a range of negative mood states and increasing some facets of positive mood. Significant differences between the active and control group were found for fluctuations in current mood measured by Visual Analogue scales (VAS), these included: alertness, content, drowsy, anxious, sadness, withdrawn, stress, and irritability. Furthermore, significant differences for both positive and negative affect in the past week were found. Negative facets of mood (anxious, withdrawn, sadness, stress, irritability, and drowsiness) were consistently improved, whereas fewer significant effects for positive mood facets were found (content and alertness). No differences were observed for perceptions of psychological health beyond affect (e.g., depression, anxiety, or stress scores), psychological wellbeing or other positive mood states such as happiness. For the second pilot experiment, no significant main effects of condition were observed, across any of the key measures (mood, affect, cognition or perceptions of psychological health).

The results from the first pilot study highlight the potential of acute supplementation with L-methylfolate at the 400mcg level, which aligns with the more widely accepted RNI (NIH, 2022). The first pilot study identified reductions in anxious, sad, irritable, stressed, and withdrawn mood for the 400mcg L-methylfolate condition, compared to the control. The consistent impact on negative emotional states could be explained by the biological anti-depressive action of folate (Stahl, 2008); and these significant reductions in negative mood support previous findings (Kennedy et al., 2010). In addition, significantly higher positive affect and content mood were reported after a single 400mcg dose of L-methylfolate, which aligns with previous non-clinical research that has found a link between higher serum folate levels and increased positive affect across eighteen months (no link was found for negative affect) (Edney et al., 2015). Significant effects for perceived stress or anxiety (DASS-21 scores) over the past week were not found. Previous folate supplementation studies have observed reductions in perceived stress, but these involve consumption over the course of weeks or months (Long & Benton, 2013; Young et al., 2019). Moreover, no differences between conditions were found for symptoms of depression (measured by the DASS-21). Consumption of highly bioavailable folate at the RDI, may rapidly optimise nutrient status and lead to specific, more immediate effects on affect (positive and negative) and mood, with no effects on perceptions of longer-term psychological health. These initial findings support and extend previous literature through identifying the isolated effects of folate in young healthy adults, separate from previous significant effects observed for folate as a component of multi-nutrient supplementation (Kennedy, 2016).

Due to COVID-19 social restrictions, recruitment was cut short and this resulted in the minimum sample size for pilot study 2 not being met (minimum sample size = 159, tested n = 65). Consequently, there were unequal group numbers across the three conditions, with the smallest number of participants being allocated to the 400mcg condition, and a similar total sample size to the first pilot (control vs. 400mcg, n = 60), thus the statistical power of the second pilot is inadequate. However, the results did suggest some evidence to support a dose-response pattern for L-methylfolate through simple evaluation of the direction of the means. These showed that negative emotional states including negative affect, sad, withdrawn, and irritable mood all decreased as the L-methylfolate dose increased, and that happy, content, and inspired mood all increased as the L-methylfolate dose increased. Furthermore, as L-methylfolate dose increased, the mean cognitive failures score decreased, whereas wellbeing scores (WEMWBS) increased, especially for the 400mcg condition compared to the control. Therefore, there is a promising trend that shows some consistency with the first pilot study with regard to reduced sad, withdrawn, and irritable mood.

Regarding the cognitive results, the significant effects of 400mcg of L-methylfolate on increased alertness, and decreased drowsiness in the first pilot study, may represent more readily detectable fluctuations in general cognitive arousal. Acute improvements to alertness and reduced drowsiness have been found in other studies that included supplementation with 400mcg of folic acid (Kennedy et al., 2010, 2011). The second pilot's results for cognition (delayed-word recall and cognitive failures scores) were non-significant, but suggested signs of improvement as the L-methylfolate dose increased. However, manipulation effects may be constrained by the baseline cognitive margins for improvement in the young adult sample, and the need for statistical power to detect any subtle deficits. Thus, it is not appropriate to draw firm conclusions without further data. Overall, the acute effects of folate manipulation on cognition in younger adults may not be detectable in this early life stage, as such individuals may not have large enough performance margins for improvement (Young et al., 2022). Alternatively, research suggests that choline and omega3-fatty acids (DHA) in combination with methyl donors such as folate, are required to improve memory scores, due to their synergistic function (Bekdash, 2019).

The strengths of the pilot experiments include the covert manipulation of L-methylfolate, as all conditions appeared to only involve the consumption of a drink of water. This blind approach therefore avoided any response expectancies which is a major limitation in previous vitamin supplementation studies (America & Milling, 2008). However, the research was posed to participants as an exploration of the effects of hydration on wellbeing, so this cover story may have influenced participant responses after consuming a glass of water, potentially causing a placebo effect (Bootzin & Bailey, 2005). To account for this, baseline thirst was included as a covariate in the analysis (ANCOVAs), but not when Area Under the Curve scores were used as the dependent variable, as this already considers fluctuations from individual baseline across the experiment. A key limitation is that the target sample sizes were not met, thus the acute effects of L-methylfolate are likely to be underpowered, and consequently, we may be unable to detect more subtle differences. For instance, the null results for psychological wellbeing (WEMWBS) in pilot study 2 may have been due to a small sample size as the p value was verging on significance and the direction of the means suggested a dose-response relationship, with the largest average wellbeing scores seen in the 400mcg condition. Collectively, the effect sizes in pilot 1 for the significant findings ranged between medium to large (mean Cohen's *d* = .81). The large effect sizes were observed for negative mood ratings (anxious, sad, withdrawn, stressed, irritable), while negative affect showed a medium effect size. For pilot 2, no significant effects emerged, and all of the eta squared values were very small, and all of the observed power was well below 80%. Consequently, for a medium effect size, pilot study 2 was underpowered (minimum sample size = 159, tested n = 65), and even though significant effects (some with large effect sizes) were found in pilot study 1, the minimum sample size (n = 102) was not met (n = 60). However, the overall minimum sample size for a large effect size when comparing the active and control group is much smaller (n = 42), thus pilot study 1 could be considered powered to detect these.

The current mood states were measured as fluctuations throughout the experiment over a duration of forty-five or sixty minutes (VAS Area Under the Curve). A single dose of L-methylfolate may impact acute fluctuations in mood, which could accumulate over time if supplementation is sustained on a daily basis, and this in turn could benefit psychological health, however, this requires future research. Thus, future adequately powered research could involve measurement of psychological health (mental health and positive psychological wellbeing), over acute periods of time (within-day, across a week and longer e.g., two weeks) using sensitive measures.

5.5 Conclusion

As dietary intake falls short of nutritional needs, insufficient folate intake may become more prevalent. These pilot findings provide early proof-of-concept for potential acute benefits of L-methylfolate (a single 400mcg dose) on negative facets of mood including reduced drowsy, anxious, and sad mood; lower feelings of being withdrawn, irritable and stressed compared to the control condition, in addition to enhanced alertness, content mood and increased positive and decreased negative affect scores. However, null effects were seen across pilot study 2 which may have been due to inadequate power to detect effects. The preliminary results support further investigation of L-methylfolate supplementation as a method for optimising folate status, mood, and affect, which in turn could protect psychological health. Oftentimes, habitual supplement consumption is a preferred solution to nutritional improvements, and considering widespread inadequate folate intake, future work could compare folate consumption in supplement or food form, as a potential avenue for improving mood, affect, cognition, and psychological health.

Chapter 6 The Acute Effects of a Vegetable-based Meal and a L-methylfolate drink on Mood, Affect, Cognition, and Psychological Health in Healthy Adults; A Randomised-Controlled Experiment

The evidence from this thesis thus far, recognises the distinct impact of fruits and vegetables on psychological health. Initially, the systematic review within Chapter 2 identified evidence to suggest a preferential effect of vegetable consumption compared to fruit for psychological wellbeing. This was shown by stronger, more robust associations and consistent benefits of vegetable consumption for both men and women, compared to fruit consumption. Within Chapter four, three significant relationships for vegetable consumption (portions and frequency) with psychological health were found across studies 2 and 3. Cross-sectionally, higher portions of vegetables predicted reduced symptoms of depression and increased positive psychological wellbeing. Prospectively, a relationship emerged for the frequency of vegetable consumption and lower symptoms of depression, after four months. Specifically, leafy green vegetables (according to previous studies identified in Chapter one's narrative review) may be relevant to psychological health because of their folate content, a micronutrient known to impact biological mechanisms that can enhance mood (Stahl, 2008). In general adult populations, daily intake of vegetables is low (Statistics on Obesity, Physical Activity & Diet, England, 2020), and this is supported by the average intake seen in the research samples in this thesis. Thus, investigating the effects of consuming folate via food (spinach) and bioavailable supplement form (L-methylfolate) warrants investigation. During the previous chapter, two pilot studies provided some evidence that L-methylfolate may reduce negative mood and affect, while increasing content mood and positive affect. The following chapter completes the final aim of the thesis, that was to experimentally explore the effect of folate (in supplement and food form) on mood, affect, cognition, and psychological health in healthy adults in a controlled laboratory setting.

6.1 Introduction

Nutrients from supplements have been shown to optimise brain function and provide neuroprotective effects (Owens et al., 2020a). Research suggests that single nutrients may be more effective than multi-nutrient supplementation for psychological health (Sarris et al., 2019). One mechanistic hypothesis is that micronutrients, such as folate play a pivotal role in the relationship between FVI and psychological health (Brookie et al., 2018; Kaplan et al., 2007; Young, 2007). Thus, L-methylfolate (highly bioavailable folate) has the potential to have a positive impact, however, it is not clear how this might compare to the equivalent consumption through food (e.g., leafy green spinach), or as a supplement on top of vegetable consumption (i.e., combined). Experiments in a laboratory setting are required to examine this as a possible mechanism in the relationship between nutrition and psychological health (Adan et al., 2019; Kris-Etherton et al., 2021).

Collectively, research shows that a positive relationship between vegetable consumption and psychological health exists (Glabska et al., 2020), this may be driven, at least in part, by folate. For instance, increasing vegetable consumption over an eight-week period has been shown to increase subjective happiness (De Leon et al., 2022). However, a sixty-day randomised trial manipulating green juice intake (containing green cabbage leaf, lettuce leaf, cucumber, and apple) compared to a placebo control resulted in increased circulating folate levels, but no significant differences between groups for wellbeing or anxiety scores (Chiochetta et al., 2018). Research shows that natural dietary folate intake has a protective, prospective effect on psychological health in healthy, non-depressed men (Tolmunen et al., 2004); whereby those with a dietary intake below 256mcg a day (compared to above) were at three times higher risk of a depression diagnosis during a follow up period (13 years on average). This relationship remained after adjustment for socioeconomic status, baseline depression score, fibre, total fat, and vitamin C intake. Only 25% of the participants reached the RNI for folate, which suggests that supplementation to improve folate status could have protective benefits. However, relying on folate consumption through vegetables is unlikely to result in enhanced nutritional status, as we know that increasing vegetable consumption is difficult due to food preferences, lack of time, access/availability, cost, and taste (Appleton et al., 2017).

L-methylfolate supplementation may offer an alternative to improving folate status through vegetables, notably L-methylfolate is naturally occurring compared to synthetic folic acid, and so this biologically active form of folate is closer to natural folate found in vegetables (Barnett, 2017). Supplementation may have more potent effects than folate-rich vegetables (e.g., spinach) as evidence shows that folic acid supplements provide greater elevation of serum folate levels than dietary intake (Elkin & Higham, 2005). This may be due to poorer bioavailability of food folate, or the presence of other foods which may hinder absorption, the sensitivity of folate to cooking, and the variability of the content of folate in foods (McNulty et al., 2019). Indeed, the method of consumption is relevant to nutrient preservation, and whether F&V is consumed raw, cooked, or from cans can impact the relationship between FVI and mental health (Brookie et al., 2018; El Ansari et al., 2014; Mikolajczyk et al., 2009). However, it is unclear whether food and supplement derived folate interact to produce more profound effects. Attempts to increase vegetable consumption alone are unlikely to have a large effect on psychological health, but effects could be amplified when combined with supplementation (Owens et al., 2020b). Experimental work is required to examine the influence of folate (in food or bioactive supplement form) as a mechanism for improving mood, affect, cognition, and psychological health. This requires the use of a supplement dose at a matched, realistic quantity for consumption through vegetables, aligned with RNI (200mcg).

Cognitive processes that tend to decline with age, namely memory, respond positively to folic acid supplementation (800mcg daily for 3 years) (Durga et al., 2007). Conversely, folate intake below the recommended daily intake is a suspected risk factor for mild cognitive impairment that increases the risk of dementia (Agnew-Blais et al., 2015). Thus, restoring folate status could improve, or slow the degradation of cognitive health (e.g., memory). Very few studies have explored the acute effects of folate consumption on memory performance after a single dose (Macpherson et al., 2015). Some studies (discussed in the introduction of Chapter 5) have reported improvements to cognitive function associated with higher dietary folate intake or multi-vitamin B supplementation over time (Bryan et al., 2002; Haskell, 2010; Kennedy, 2010; Qin et al., 2017). Folate consumed in supplement and/or food form may have acute effects on the cognitive failures reported by healthy adults, but this is yet to be explored experimentally.

To our knowledge, no research to date has explored the acute within-day effects of folate via a vegetable-based meal and/or L-methylfolate supplementation on mood, affect, cognition and psychological health. Plasma folate has shown to significantly increase 1-2 hours after consumption of a spinach-containing meal (300-600g of spinach) and for up to 6 hours after (Brönstrup et al., 1998). Also, research has shown that consuming a B vitamin supplement significantly increases circulating folate via biological measures within 24 hours (Drogan et al., 2004). More broadly, micronutrients have physiological effects within 1-3 hours of ingestion (Haskell et al., 2008; Scholey et al., 2013; White et al., 2015). Thus, a bioavailable L-methylfolate drink and/or spinach-based meal may result in detectable psychological effects after absorption; this study will explore whether effects occur within 75 minutes.

Thus, this 2x2 between-subject design, randomised-controlled experiment assessed the acute effect of consuming the RNI (200mcg) of folate in vegetable and/or supplement form, on mood, affect, cognition and psychological health compared to controls. The manipulation involved a meal, with or without spinach, and a drink, with or without L-methylfolate. It was hypothesised that positive psychological outcomes would be highest when participants consumed the vegetable-based meal and the L-methylfolate drink, whereas positive psychological outcomes would be lowest when participants consumed the non-vegetable-based meal and the placebo drink (water). Additionally, it was hypothesised that negative psychological outcomes would be lowest when participants consumed the vegetable-based meal and the L-methylfolate drink, whereas negative psychological outcomes would be highest when participants consumed the non-vegetable-based meal and the placebo drink (water). We also hypothesised that the L-methylfolate drink would show a superior effect compared to the consumption of the vegetable-based meal, due to its bioavailability.

6.2 Method

Participants

Healthy adult participants (staff and students) were recruited through posters placed around Aston University campus, via emails circulated using departmental newsletters, and an advert on the Research Participation Scheme (RPS) website. Participants received either a £15 Amazon voucher in exchange for their time, or RPS credits plus a £5 Amazon voucher. The experiment was advertised as a study of 'Lifestyle Habits, Wellbeing and Cognition.' Participants completed an initial screener questionnaire via email correspondence to ensure they met the inclusion criteria. Participants who rated their general health as poor over the last 12 months, who currently have or had diabetes, or an eating disorder and/or medically diagnosed food allergy, high blood pressure, a heart attack, or were experiencing medical illness were not eligible to participate. Participants were asked to refrain from eating food for 2 hours prior and not to consume any caffeine 24 hours beforehand. A total of 172 adults expressed an interest to take part, with 108 participants completing the experiment (32 were not eligible to take part, 23 participants did not show for the experiment, 7 refused to consume the meal, and 2 consumed caffeine before participating, and thus, were excluded during the eligibility check upon arrival). The experiment was approved by the College of Health & Life Sciences Ethics committee at Aston University.

Sample size

Using G*Power, with number of groups set to 4, alpha set at 0.05, modelling a medium effect size (*d* = 0.5), and power at 80%, the minimum required sample size was 204 participants overall (51 per group) (Faul et al., 2009). The minimum sample size was not achieved (tested n = 108) because recruitment was prematurely terminated due to the global COVID-19 pandemic and lock-down restrictions, and then a leave of absence was taken for maternity leave.

Design

This randomised-controlled experiment was a 2x2 between-subjects design. The between-subjects factors were drink (L-methylfolate or no L-methylfolate) and meal (vegetable and rice meal or rice meal). Thus, participants were randomly assigned to one of the four following conditions: (1) vegetable and rice meal (200 mcg folate) + L-methylfolate drink (200 mcg in 125ml of water); (2) vegetable and rice meal + control drink (125ml water); (3) rice meal + L-methylfolate drink, or; (4) rice meal + control drink. There were multiple dependent variables including: VAS, PANAS, WHO-5, WEMWBS, CFQ, DASS-21, and HADS scores. The VAS were measured at 15-minute intervals; thus, a within-subjects factor of 'time' applies to this. Participants completed one testing session for the duration of 80 minutes, and this took place in Aston University's Nutrition laboratory.

Measures

The measures used in the first and second pilot experiments were used again in this experiment, except for the delayed-word recall task was omitted, and three further additions were made:

The Hospital Anxiety and Depression Scale (HADS): The 14-item HADS (Zigmond & Snaith, 1983) was used to measure symptoms of anxiety and depression during the past week. An example item from the anxiety subscale is 'I feel tense or wound up' and from the depression subscale is 'I feel as if I am slowed down.' Participants rated items for each subscale on a 4-point scale ranging from 0 (not at all) to 3 (most of the time). Total scores were computed for anxiety and depression after reverse scoring as required. This measure was added as the sample included more generally healthy adults (between 18-63 years old), whereas in the previous pilot experiments, the DASS-21 was more appropriate for student only samples.

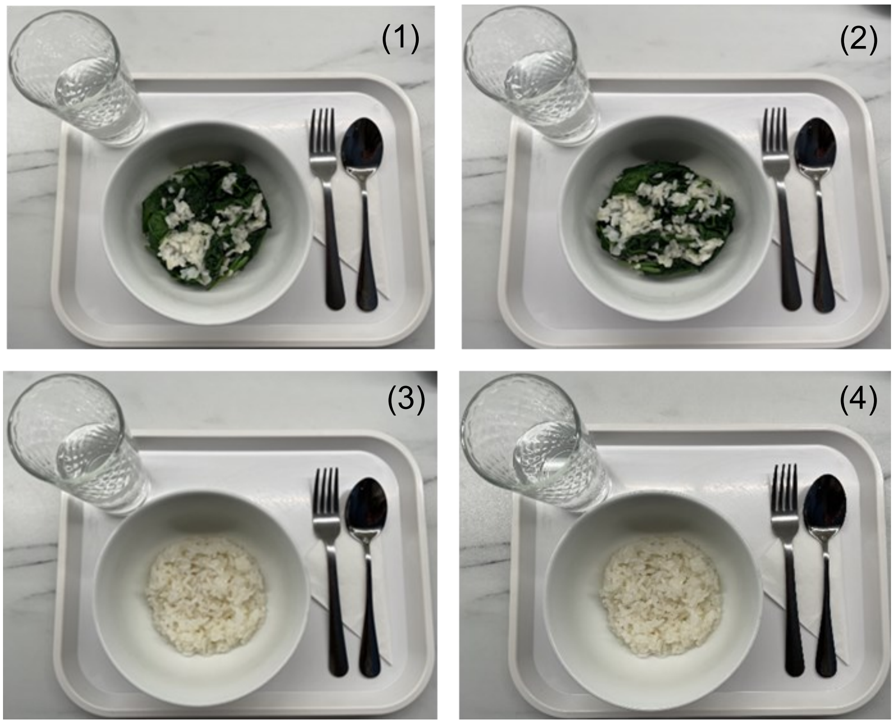
Cognitive tasks 1 and 2: These were the N-Back working memory task (Kirchner, 1958) which was conducted in E-prime. Briefly, in the N-Back task, participants were presented with a sequence of stimuli one-by-one in letter form. Participants are told to indicate (via a response on the keyboard) when the current stimuli match the one from n steps earlier in the sequence. The *N* can be 1, 2, or 3 steps back. The Stop-signal inhibitory control task ran in Matlab using the original script from Verbruggen et al., 2019 (this task was previously discussed in study 1, Chapter 3). These tasks were not analysed as part of this thesis and were treated as filler tasks between the final VAS mood ratings.

Fruit and vegetable (F&V) liking: Two items were used to measure how much participants enjoyed eating fruit and vegetables; these were used to assess for differences between participants allocated to each condition. These items followed the same format as VAS items, whereby participants rated the degree to which they enjoyed eating fruit and vegetables (separately) on a scale from 0 (not at all) to 100 (very much).

Drink and meal manipulation and preparation

Folate was manipulated in the form of the drink or the meal (matched for microgram dose), or both, compared to the control drink and meal. The drink and meal were prepared prior to the participant's arrival (see Appendix B for the preparation details). The L-methylfolate drink was 125ml of filtered water presented in a glass with 2 drops of L-methylfolate (200mcg of folate), and the control drink was 125ml of filtered water. The vegetable and rice meal were made from the following ingredients: 76 grams (g) of cooked rice (30.4g uncooked), 137g of pre-washed spinach leaves, 8g of margarine and 0.5g of salt. The rice meal was made from the following ingredients: 76g of cooked rice, 8g of margarine and 0.5g of salt. To account for the processing of spinach (use of low heat), FoodData (Agricultural Research Service, 2018) was consulted, which suggested that 137g of pre-washed spinach leaves represents 200mcg total folate once cooked (1.46mcg per gram). During piloting (see Appendix C for the rationale for piloting spinach as the vegetable) this amount created a realistic portion, accepted as part of a vegetable-based meal. The meal was served in a white bowl, and the drink in a transparent glass, along with a fork, spoon, and serviette presented on a white tray, see Figure 6.1 showing the presentation of each condition.

Figure 6.1 The presentation of the drink and meal for each condition.



Note: (1) Vegetable and rice meal + L-methylfolate drink; (2) Vegetable and rice meal + control drink (125ml water); (3) A rice meal + L-methylfolate drink; (4) A rice meal + control drink.

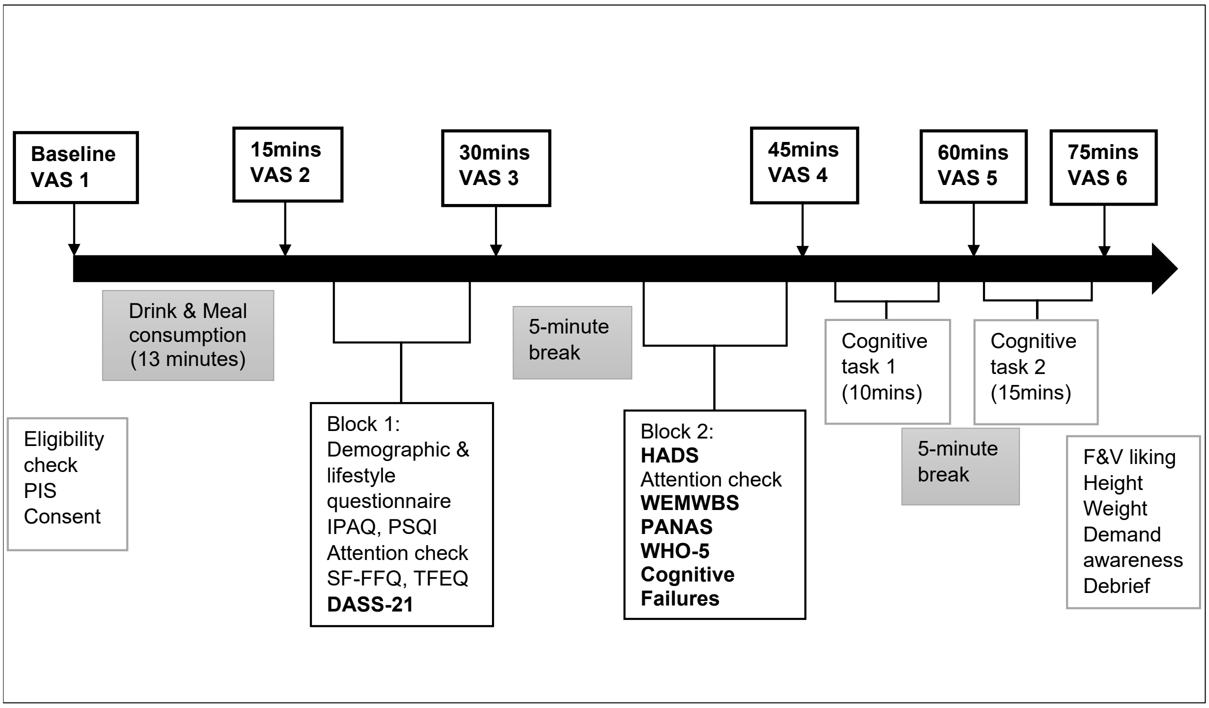
Procedure

Participants who expressed a willingness to take part via email correspondence or through the University RPS were sent an email with the initial screener questionnaire and made aware of the inclusion criteria. Eligible participants were told to not consume caffeine 24 hours prior to the experiment and to consume food no later than 2 hours before attending the laboratory. Testing sessions took place between early to late lunch time (11am-3:30pm) due to the consumption of a meal. Upon arrival at the laboratory, a final eligibility check was completed, and participants were sat at a computer to record their responses within Qualtrics, a web-based software for hosting surveys. Firstly, participants read the information sheet (which had also been emailed to them at least 24 hours before). Following this, participants provided informed consent and completed their baseline mood (VAS) ratings. Participants were then presented with a drink and meal on a tray (according to their random allocation via the Excel random selection formula) and left to consume this for 13 minutes. Participants were also provided with a doorbell to alert the experimenter when they had finished, or if they needed attention for health and safety reasons etc.

After the drink and meal were consumed, participants were asked to complete a VAS mood rating directly after, followed by the first block of questionnaires which involved completion of a demographic and lifestyle questionnaire, then the IPAQ, PSQI, an attention check, followed by the SF-FFQ and TFEQ. Then, the DASS-21 was completed before participants were instructed to complete their third VAS mood rating, followed by a 5-minute break (they were not allowed to go on their phone and were told to read the magazines). Participants were then asked to complete the second block which involved completion of the HADS, an attention check, the WEMWBS, PANAS, WHO-5 and cognitive failures questionnaire. Then, after the fourth VAS mood rating at 45 minutes, participants completed the first cognitive task (10-minute N-Back task) and following this were instructed to take another 5-minute break. Participants then completed the fifth VAS mood rating at 60 minutes, followed by the second cognitive task (15-minute Stop-signal task). Finally, the sixth VAS mood rating was completed at 75 minutes and the final block involved rating how much the participant liked fruit and vegetables, then participants' height and weight were measured using a stadiometer and weighing scales. To end, the participants were debriefed, the aims of the research were clarified, and as appropriate, it was explained to participants that they had consumed their daily intake of folate in liquid form via the drink of water, or food form via the meal.

The total duration of the experiment was approximately 80-85 minutes (mean duration = 81 minutes). The key measure of interest (VAS mood ratings) was completed at 15-minute intervals a total of six times throughout the experiment, and timing was measured throughout to maintain the procedural order (see Figure 6.2). When required, the filler activity of asking participants to read neutral magazines was implemented to ensure consistent timings across experiments. Furthermore, depression and anxiety were measured twice using the DASS-21 and HADS, as these are appropriate for university students and the general adult population (staff and student sample). Wellbeing was also measured twice using the WHO-5 and WEMWBS to allow for comparison.

Figure 6.2 Experiment 3 procedural order.



Note: Measures in bold text and within the blocks represent the key measures. Measure abbreviations: Participant Information Sheet (PIS), World Health Organisation-5 item wellbeing scale (WHO-5), Visual analogue scales (VAS), Short-form food frequency questionnaire (SF-FFQ), Three-factor eating questionnaire (TFEQ), International physical activity questionnaire (IPAQ), Pittsburgh sleep quality index (PSQI), Positive and negative affect schedule (PANAS), Depression anxiety stress scale (DASS-21).

Statistical analyses

Participant characteristics and baseline scores: were assessed using one-way ANOVAs to determine whether significant differences between the four conditions existed at the beginning of the experiment. Descriptive data were calculated as means with standard deviation, or as frequencies (%) for categorical variables.

Covariate analysis to inform ANCOVA analyses: Pearson's correlations for theoretical covariates revealed that general health rating was significantly correlated with four aspects of psychological health, this included positive and negative affect PANAS scores, wellbeing scores (both WEMWBS and WHO-5) and depression *ps* <.05), thus was included in the following ANCOVA analyses. Additionally, household income was significantly negatively associated with anxiety score, and positively with wellbeing (WEMWBS) score (*ps* <.05). Also, baseline leafy green vegetable intake was included as a covariate, upon evaluation this was significantly, positively associated with wellbeing scores (both WEMWBS and WHO-5) and positive affect PANAS score (*ps* <.05). As this experiment involved the manipulation of folate consumption (meal or L-methylfolate form), baseline leafy green vegetable intake was considered a proxy for folate status, which is relevant for accounting for individual margins for improvement. Finally, age was included as a covariate because this was significantly negatively associated with anxiety scores (DASS-21 and HADS) and positively with cognitive failures score (*ps* <.05). None of the other theoretical measures correlated with at least two of the outcomes, thus were not included as covariates. Sleep duration and physical activity minutes per week were both significantly, positively correlated with only WHO-5 wellbeing score. However, the included covariate of general health rating is likely to indirectly capture sleep and physical activity, as both are independently correlated with general health rating (Memon et al., 2021).

Main analyses: Due to the wellbeing (WHO-5) and mood (VAS) measures being completed at multiple time points, the data were converted into an Area Under the Curve (AUC) score to allow for total fluctuations as a function of time to be studied. The AUC score was computed using the trapezoidal rule method (Le Floch et al., 1990) which considered the fluctuations in scores from baseline through to the last measure taken at 75 minutes. A spreadsheet in Excel was used to apply the integral formula and calculate each AUC score. The analysis involved two-way ANOVA, and ANCOVA analyses with covariates, to identify any significant main effects for meal and drink (and any interaction), for mood ratings, affect, cognitive failures and perceived psychological health including depression, anxiety and stress measured by both the DASS-21 and HADs, and positive wellbeing measured by the WEMWBS and WHO-5. Finally, follow up exploratory analysis was conducted using independent samples t-tests to examine the difference between the control (no folate consumption) and the active (maximum folate consumption) conditions: rice-meal with plain water (0mcg) vs. vegetable-meal with L-methylfolate drink (400mcg) for mood ratings (VAS) scores. One-way ANCOVA analyses were conducted including general health rating, household income, age, and leafy green vegetable intake (daily portions) as covariates, to examine the difference between the control and active condition on psychological health ratings.

6.3 Results

Participant characteristics and baseline scores

One-hundred and eight participants completed the experiment with an overall mean age of 24.22 (SD = 8.04, range = 18 to 63 years old), 72% were female (n = 78) and 28% (n = 30) were male. The participants' ethnic background was as follows: 34% Asian, 44% White, 14% Black, 3% Chinese, and 5% were mixed ethnicities. Participants' average BMI was within a healthy range (mean = 24.2, SD = 4.88). Most of the participants' household income for the past 12 months (before taxes and from all sources) was between £10,000 and £49,999. Six percent were smokers and 38% drank alcohol. Sixty-one percent of participants averaged 7+ hours of sleep per night, and ten percent engaged in vigorous (e.g., heavy lifting or aerobics) physical activity once a day. For the overall sample, the DASS-21 and HADs scores indicated a non-clinical sample (DASS-21 mean: depression = 11.0 (SD = 4.1), anxiety = 10.4 (SD = 3.3), stress = 12.6 (SD = 3.8); HADs mean: anxiety = 7.3 (SD = 4.3), depression = 4.1 (SD = 3.0).

Further descriptive data by condition, is presented in Tables 6.1 and 6.2. At baseline, there were no significant differences between conditions for demographic, lifestyle, and mood ratings (see Table 6.1), nor were there any significant differences between condition for BMI, eating habits (daily portions) or eating style (see Table 6.2). For food frequency (SF-FFQ), on average, participants consumed fruit 2-3 times a week, vegetables 4-6 times a week, and sweet and savoury snacks 2-3 times a week. Overall, participants consumed on average 1.75 portions (SD = 1.2) of fruit a day, 1.86 portions (SD = 1.2) of vegetables a day, and .98 portions (SD = .98) of leafy green vegetables (spinach, broccoli etc.). For information regarding participants' eating habits, see Table 6.2.

Table 6.1 Baseline demographic, lifestyle behaviours, and mood ratings by condition.

N, mean (SD or %)	**Vegmeal+ L-methylfolate n = 25**	**Vegmeal+ water n = 25**	**Ricemeal+ L-methylfolate n = 31**	**Ricemeal+ water n = 27**	***p***
Demographic					
Sex (%)					
Female	16 (64)	17 (68)	24 (76)	21 (78)	
Male	9 (36)	8 (32)	7 (24)	6 (22)	
Mean age (SD)	27.0 (10.0)	24.5 (10.0)	22.8 (5.4)	23.0 (6.1)	.198
Household income	20-49K	20-49K	20-49K	20-49K	.523
Lifestyle behaviours					
Physical activity total minutes last week (SD)	83.6 (80.7)	75.0 (111.1)	107.9 (154.0)	93.3 (122.1)	.776
Sleep Duration (SD)	6.6 (2.2)	6.6 (2.1)	5.6 (2.8)	6.4 (2.5)	.450
Alcohol intake (units per week)	<14 units	<14 units	<14 units	<14 units	.988
Smoking status (cigarettes total)	<100	<100	<100	<100	.714

General Health Rating	Very good	Very good	Very good	Very good	.114
Baseline Mood Ratings (VAS)					
Calm	72.8 (22.6)	70.8 (21.0)	74.6 (18.3)	59.5 (28.3)	.065
Hungry	53.0 (30.3)	59.6 (23.9)	65.3 (24.6)	60.4 (30.3)	.426
Thirsty	58.2 (24.6)	68.1 (16.8)	60.2 (25.2)	54.4 (28.4)	.235
Desire to Eat	57.9 (30.8)	56.4 (27.0)	64.4 (26.2)	59.2 (27.9)	.721

Note: n = Number. SD = Standard Deviation

Table 6.2 Participants' Body Mass Index (BMI), eating habits and style by condition.

Mean (SD)	**Vegmeal+ L-methylfolate n = 25**	**Vegmeal+ water n = 25**	**Ricemeal+ L-methylfolate n = 31**	**Ricemeal+ water n = 27**	***p***
BMI	25.6 (6.5)	22.3 (3.2)	23.6 (4.3)	25.2 (4.5)	.055
Fruit intake (portions)	1.9 (1.2)	1.6 (1.0)	1.9 (1.4)	1.5 (1.1)	.373
Vegetable intake (portions)	1.8 (1.3)	1.8 (1.4)	1.8 (1.1)	2.0 (1.1)	.860
Leafy green vegetable intake (portions)	0.9 (0.8)	0.9 (1.3)	1.1 (1.0)	0.8 (0.7)	.685
Fruit liking (0-100)	80.9 (18.3)	79.7 (14.2)	82.9 (17.2)	80.9 (24.2)	.936
Vegetable liking (0-100)	69.6 (23.9)	64.2 (29.2)	72.4 (23.2)	77.0 (22.0)	.302
Sweet snacking (portions)	0.9 (0.7)	1.3 (1.4)	1.2 (1.1)	1.6 (1.5)	.292
Savoury snacking (portions)	0.6 (0.5)	0.92 (0.5)	0.60 (0.7)	1.0 (1.4)	.137
Uncontrolled eating	2.0 (0.5)	2.0 (0.6)	2.2 (0.5)	2.3 (0.7)	.186
Cognitive restraint	2.2 (0.5)	2.1 (0.7)	2.1 (0.8)	2.3 (0.7)	.800
Emotional eating	1.9 (0.8)	1.65 (0.7)	1.89 (0.6)	2.07 (0.9)	.253

Main analyses

Area Under the Curve VAS mood scores: Two-way ANOVA analyses were conducted to evaluate meal and drink effects and their interaction on mood ratings as Area Under the Curve scores. These revealed no significant main effects or interactions (all *ps*>.05). Please see Table 6.3 for the means and SD by meal and drink condition.

Table 6.3 Means and standard deviation (SD) of mood ratings (VAS 1-6, baseline to 75 minutes) Area Under the Curve scores by meal and drink (two-way ANOVA).

Mean (SD)	**Vegmeal+ L-methylfolate n = 25**	**Vegmeal+ water n = 25**	**Ricemeal+ L-methylfolate n = 31**	**Ricemeal+ water n = 27**
Alert	4974 (1614)	4852 (1105)	4708 (1456)	4192 (1824)
Drowsy	1970 (1671)	2079 (1747)	2180 (1625)	2075 (1686)
Anxious	917 (1315)	1181 (1370)	949 (1199)	1577 (1977)
Happy	5013 (1476)	4304 (1488)	4412 (1500)	4080 (1619)
Sad	613 (968)	1036 (1290)	913 (1532)	1563 (1746)
Withdrawn	1012 (1317)	1194 (1294)	1103 (1342)	1641 (2032)
Stress	1374 (1634)	1645 (1744)	1669 (1901)	2149 (2277)
Content	4977 (1615)	4504 (1886)	4366 (1595)	4052 (1656)
Inspired	3190 (1983)	2493 (1890)	3422 (1724)	3042 (2038)
Calm	5249 (1891)	5189 (1462)	5076 (1659)	4953 (1718)
Irritable	1109 (1526)	1162 (1385)	1404 (1348)	1560 (1651)

Subjective psychological health ratings, affect, and cognition: Two-way ANCOVA analyses revealed significant main effects for the L-methylfolate drink on psychological health ratings (perceived anxiety and negative affect scores). This analysis included the following covariates: general health rating, household income, age, and leafy green vegetable intake (daily portions). For anxiety scores (DASS-21, measured once at 28-30 minutes), there was a significant main effect of L-methylfolate drink, ($F(1,100) = 5.211$, $p = .025$, $\eta^2 = .050$, observed power = .618) with participants that consumed L-methylfolate reporting significantly lower anxiety scores than those who did not (means = 9.67 vs. 11.01, respectively). However, there was no significant main effect of meal ($F(1,100) = 3.175$, $p = .078$, $\eta^2 = .031$) or interaction effect of drink and meal ($F(1,100) = .063$, p = .803, $\eta^2 = .001$). The means for anxiety scores measured by the HADs followed a similar pattern to the DASS-21, but there were no significant main effects or an interaction (*p*s > 0.05). For negative affect (PANAS, measured once at 40-42 minutes), there was a significant main effect of L-methylfolate drink, ($F(1,100) = 4.110$, $p = .045$, $\eta^2 = .039$, observed power = .519), with participants that consumed L-methylfolate reporting significantly lower negative affect scores than those who did not (means = 17.75 vs. 20.26, respectively). However, there was no significant main effect of meal ($F(1,100) = 1.389$, $p = .241$, $\eta^2 = .014$) or interaction effect of drink and meal ($F(1,100) = .079$, p = .779, $\eta^2 = .001$). There were no further significant main effects for drink or meal, or interaction results for any of the other cognition and psychological health ratings (*ps*>.05). The means and SD by condition are presented in Table 6.4.

Table 6.4 Means and standard deviation (SD) of cognition, affect, and psychological health ratings by meal and drink condition (two-way ANCOVA).

Mean (SD)	Vegmeal+ L-methylfolate n = 25	Vegmeal+ water n = 25	Ricemeal+ L-methylfolate n = 31	Ricemeal+ water n = 27
Cognitive Failures	37.56 (12.03)	38.96 (16.08)	42.29 (17.39)	42.26 (14.06)
Depression (DASS-21)	10.72 (3.70)	11.72 (4.87)	10.16 (3.60)	11.67 (4.27)
Anxiety (DASS-21)	8.92 (2.45)	10.20 (2.57)	10.23 (2.88)	11.96 (4.45)
Stress (DASS-21)	12.20 (3.49)	12.56 (3.37)	12.39 (3.98)	13.22 (4.25)
Depression (HADS)	4.28 (3.08)	4.08 (3.49)	3.68 (2.60)	4.63 (2.95)
Anxiety (HADS)	6.12 (3.38)	7.48 (3.85)	7.58 (4.68)	8.04 (4.99)
Positive Affect (PANAS)	31.48 (6.51)	31.20 (7.92)	32.71 (7.33)	32.37 (7.57)
Negative Affect (PANAS)	16.72 (4.89)	19.04 (6.75)	18.81 (5.69)	21.37 (8.11)
Wellbeing (WEMWBS)	49.0 (8.0)	47.08 (8.23)	47.55 (10.95)	47.41 (9.88)
Wellbeing (WHO-5)	14.68 (4.64)	13.80 (5.45)	14.97 (5.38)	13.96 (5.75)

Note: Covariates = general health rating, household income, age, and leafy green vegetable intake (daily portions).

Exploratory analysis

Area Under the Curve VAS mood scores: Independent samples t-tests were conducted to examine the difference between the control (no folate consumption) and the active (maximum folate consumption) conditions: rice-meal with plain water (0mcg) vs. vegetable-meal with L-methylfolate drink (400mcg) for mood ratings (VAS) scores. These revealed three significant differences (all *ps* <.05) for happy, content, and sad AUC mood scores (medium effect size on average, Cohen's d = .6). For positive mood (happy and content), higher mood ratings were observed in the active condition compared to the control. Conversely, sad mood was lower in the active condition compared to the control. There were no further significant differences in mood ratings. Please see the following Table 6.5, for the statistical values.

Table 6.5 Independent t-test results for mood ratings (VAS 1-6, baseline to 75 minutes) Area Under the Curve scores by condition.

	Control	**Active**	
	n = 27	**n = 25**	
	Rice-meal + water	**Veg-meal + L-methylfolate**	
	Mean (SD)	**Mean (SD)**	***p***
Alert	4192 (1824)	4974 (1614)	.054
Drowsy	2075 (1686)	1970 (1671)	.411
Anxious	1577 (1977)	917 (1316)	.083
Happy	4080 (1619)	5013 (1476)	.018*
Sad	1563 (1746)	613 (968)	.010*
Withdrawn	1641 (2032)	1012 (1317)	.098
Stressed	2149 (2277)	1374 (1634)	.084
Content	4052 (1656)	4977 (1615)	.024*
Inspired	3042 (2038)	3190 (1983)	.397
Calm	4953 (1718)	5249 (1890)	.279
Irritable	1560 (1651)	1109 (1525)	.156

Note: * p <.05. Rice-meal+ water= 0mcg of folate, Veg-meal + L-methylfolate= 400mcg of folate.

Subjective psychological health ratings, affect, and cognition: To examine the difference between the control (no folate consumption) and the active (maximum folate consumption) conditions, with the inclusion of covariates, one-way ANCOVA analyses were conducted including covariates: general health rating, household income, age, and leafy green vegetable intake (daily portions). For negative affect (PANAS), there was a significant main effect of condition (F(1,46) = 4.172, p = .047, η^2 = .083, observed power = .516), whereby those in the active condition scored lower for negative affect compared to those in the control condition (see Table 6.6 below for the means and SD). However, for positive affect (PANAS) scores, there was no significant main effect for condition (F(1,46) = .660, p = .421, η^2 = .014, observed power = .125). Also, for anxiety scores measured by the DASS-21, there was a significant main effect of condition (F(1,46) = 5.890, p = .019, η^2 = .114, observed power = .661), whereby those in the active condition scored lower for anxiety compared to those in the control condition (see Table 6.6). However, anxiety scores measured by the HADS showed no significant main effect of condition (F(1,46) = .930, p = .340, η^2 = .020, observed power = .157). For cognitive failures scores, there was no significant main effect for condition (F(1,46) = .385, p = .538, η^2 = .008, observed power = .093), and this was also the case for depression scores measured by the DASS-21 (F(1,46) = .126, p = .724, η^2 = .003, observed power = .064), and measured by the HADS (F(1,46) = .004, p = .951, η^2 = .000, observed power = .050). For stress scores, there was no significant main effect for condition (F(1,46) = .119, p = .731, η^2 = .003, observed power = .063). Furthermore, no significant main effect for condition was found for wellbeing scores measured by the WEMWBS ($F(1,46) = .005$, $p = .943$, $\eta^2 = .000$, observed power = .051), or the WHO-5 ($F(1,46) = .010$, $p = .921$, $\eta^2 = .000$, observed power = .051).

Table 6.6 Means and standard deviation (SD) of cognition, affect, and psychological health ratings by condition (one-way ANCOVA).

	Control	**Active**
	Rice-meal + water	**Veg-meal + L-methylfolate**
Measure	**Mean (SD)**	**Mean (SD)**
Cognitive Failures	42.26 (14.06)	37.56 (12.03)
Depression (DASS-21)	11.67 (4.27)	10.72 (3.70)
Anxiety (DASS-21)	11.96 (4.45)	8.92 (2.45)*
Stress (DASS-21)	13.22 (4.25)	12.20 (3.49)
Depression (HADS)	4.63 (2.95)	4.28 (3.08)
Anxiety (HADS)	8.04 (5.0)	6.12 (3.38)
Positive Affect (PANAS)	32.37 (7.57)	31.48 (6.51)
Negative Affect (PANAS)	21.44 (8.11)	16.72 (4.89)*
Wellbeing (WEMWBS)	47.41 (9.88)	49.0 (8.01)
Wellbeing (WHO-5)	13.96 (5.75)	14.68 (4.64)

Note: Covariates = general health rating, household income, age, and leafy green vegetable intake (daily portions), * p <.05. Rice-meal+ water= 0mcg of folate, Veg-meal + L-methylfolate= 400mcg of folate.

6.4 Discussion

This randomised-controlled experiment explored the within-day, acute effects of folate consumed as part of a spinach and rice meal, and/or, a L-methylfolate supplement drink, and their effects/interaction on mood, affect, cognition, and psychological health in healthy adults. There was a significant effect of drink for subjective anxiety scores, whereby participants that consumed L-methylfolate reported significantly lower anxiety scores and significantly lower negative affect scores over the past week, than those who did not. General health rating, household income, age, and leafy green vegetable intake (daily portions) were included as covariates in this analysis. No further effects of drink, meal, or the interaction between these were found for affect, cognition, or psychological health. Follow up exploratory analysis to examine the difference between the control (rice-meal with plain water, 0mcg folate) and the maximum folate consumption (vegetable-rice meal with L-methylfolate drink, 400mcg folate) for mood ratings (VAS) scores revealed three significant differences for happy, content, and sad mood across the time course of the experiment; higher happy and content mood ratings, and lower sadness mood ratings, both in the active condition compared to the control. No further significant effects on mood were found. Further exploratory analyses (ANCOVA) revealed that for affect and psychological health, those who consumed the maximum folate (veg-rice meal and L-methylfolate condition, 400mcg) reported lower negative affect and perceived anxiety in the past week, compared to those who did not consume any folate.

The selective effects of the L-methylfolate drink (200mcg) on the reduction of perceptions of negative emotional states (perceived anxiety and negative affect in the past week), support previous RCT findings in healthy women after the consumption of one multi-vitamin tablet containing 500mcg of folic acid (Macpherson et al., 2015). In this previous study, benefits were found to anxiety and stress scores, but no effects on symptoms of depression or cognition were seen. The significant benefits were observed one-hour post dose which aligns with the peak folate concentration for folic acid (Maki et al., 2012). Bioavailable L-methylfolate is considered to impact plasma folate concentrations after 30 minutes, peaking at 45 minutes (Finglas et al., 2003). The current effects align with this timeframe for the metabolism of L-methylfolate for negative affect and anxiety scores (28-42 minutes). Previous research shows that negative affect variability in healthy males has been shown to decrease as red blood cell folate concentrations increase, but this is a more accurate measure of long-term folate status (Williams et al., 2008). The current findings extend previous research through evaluating psychological perceptions after acute consumption of highly bioavailable folate, rather than quasi-observation or retrospective evaluation of dietary folate intake. These results suggest that the effects of L-methylfolate may be more potent than vegetable consumption for elements of psychological health.

When maximum folate from the meal and drink (400mcg) vs. no folate consumption was compared, the results replicated the effects on perceived anxiety and negative affect, and in addition, some effects on fluctuations in mood over the course of the experiment were observed (limited to this exploratory analysis). Of note, these were for both positive (happy, content) and negative (sad) mood; the first pilot study (Chapter 5) which used the same microgram dose of L-methylfolate (400mcg) also identified effects on content and sad mood. The null effects for L-methylfolate drink within the main analysis could be due to the need for a higher intake of folate (e.g., 400mcg and above), as the vegetable-based meal and L-methylfolate were matched and manipulated at 200mcg. Thus, acute fluctuations in mood may not respond to a lower 200mcg intake of folate (Papakostas et al., 2012). However, it seems more likely that the null results are due to a lack of statistical power (due to not meeting the target sample size) and thus, we are not able to detect relatively subtle changes in mood (Young et al., 2019), as a consistent pattern is seen for the direction of the means across various measures.

Longer-term supplementation duration may be required to influence symptoms of depression. A systematic review has identified that short-term use of folic acid or L-methylfolate supplementation (30 days-26 weeks) did not reduce the onset and severity of depressive symptoms (Almeida et al., 2015). Similarly, improvements to nutrient status over time may be required to influence cognition; as previous research using a battery of cognitive tasks (known to be sensitive to nutritional status) also did not observe any acute effects 1-2 hours after a single dose of a multi-nutrient supplement (including 500mcg of folic acid) on cognitive performance (Macpherson et al., 2015). Furthermore, a synthesis of studies has concluded that folic acid supplementation via RCTs (4-96 weeks in duration) show no significant effect on memory improvement in adults 18 years and older (Akhgarjand et al., 2022), whereas long-term folate blood concentration over a three-year follow-up period is positively associated with immediate recall and mental speed, and weakly associated with working memory in middle and older age (Horvat et al., 2016).

Strengths of the study include the randomised, placebo-controlled design. The manipulation of L-methylfolate was undetectable in a drink of water, which eradicated the issue of response expectancies associated with vitamin tablet consumption (America & Milling, 2008). A novel manipulation was used to explore the acute effect of consuming the RNI of folate in a vegetable meal and/or a highly bioavailable drink, on mood, affect, cognition, and psychological health. However, methodological limitations include that this experiment manipulated the low dose of L-methylfolate (200mcg), and the 400mcg level of L-methylfolate was not evaluated due to the need to match the micrograms of folate within the drink to the vegetable-based meal. The sample size target for this experiment was not met due to COVID restrictions halting data collection. Consequently, low, and unequal group sizes occurred, so interpretation of the results should take this into account. Further adequately powered research is required. Finally, the means for anxiety scores measured by the HADs, followed a similar pattern to the DASS-21, but were not significant. Both the DASS-21 and the PANAS have especially strong construct validity in non-clinical samples, so this may explain their sensitivity to detect more subtle group differences (Crawford & Henry, 2004; Sinclair et al., 2012).

Future research could explore margins for improvement to psychological health and nutritional status. As the current study involved healthy adults with non-clinical psychological health scores, future work could recruit dysphoric participants experiencing low mood (i.e., with high sadness, anxious or negative affect scores) to scrutinise margins for change and the potency of L-methylfolate effects further. Furthermore, the measurement of prior micronutrient intake e.g., the use of vitamin or mineral supplements was not assessed so baseline nutritional status could confound, conflate, or attenuate the effects of L-methylfolate due to synergistic action (Quadros, 2023); however, participants were fasted. To counter this, daily portions of leafy green vegetable consumption were used as a proxy for folate intake and included as a covariate. There is a need to consider individual differences in biological response to L-methylfolate, for instance effects may be mediated by a genetic mutation known to impair folate metabolism (Gilbody et al., 2007b), thus accounting for genetic differences could also be relevant. Additionally, individuals have unique nutritional needs and metabolic health which could have confounding effects (Adan et al., 2019).

Moreover, restoring the bioavailability of one micronutrient assumes sufficient availability of other micronutrients, which is problematic because micronutrients interact in complex biochemical pathways (Combet & Gray, 2019). Research suggests that the benefits of B vitamins may be dependent on diet quality, co-nutrient optimisation, and the interdependency of nutrients e.g., folate and vitamin B12 work synergistically (Young et al., 2022). Thus, further work could holistically evaluate the baseline nutritional status of participants, through the use of measures that enable the quantification of habitual micronutrient and antioxidant intake consumed through diet (e.g., urinary biochemical measurement). Furthermore, future acute supplementation could involve short-term provision of L-methylfolate to take home (to add to a daily drink of water) and the remote assessment of mood, affect, and perceptions of psychological health via ecological momentary assessment (EMA). Emerging evidence suggest that subtle changes to mood using in-home EMA phone-based measurement may be more sensitive in comparison to laboratory assessments (Ebner-Priemer & Trull, 2009; Macpherson et al., 2016).

6.5 Conclusion

The current results suggest that acute effects of a single dose of L-methylfolate (aligned with the UK's NHS RNI of 200mcg) via a drink may reduce perceptions of anxiety and negative affect scores. However, no effects were observed for the same amount of folate consumed through a vegetable-based meal. If reductions to negative emotional states are sustained over time through L-methylfolate supplementation, this could offer protective benefits to mental health (Al Ammar et al., 2020). Further research is required and should explore the microgram dose and duration of L-methylfolate consumption, in addition to the interaction with regular vegetable consumption for optimising folate status, mood, affect, cognition, and psychological health.

Chapter 7 General Discussion

7.1 Thesis aims and chapter findings

The aims of this thesis were firstly, to assess the role of vegetable consumption (relative to fruit) in psychological health through a systematic review of prospective data in healthy adults. The findings showed that few studies measured fruit and vegetable consumption separately (n= 3), and only ten eligible studies were identified (Chapter 2). From the limited evidence, a superior effect of vegetables (vs. fruit) on positive psychological wellbeing was observed, whereas the effects of F&V consumption on mental health were inconsistent. Hence, additional research followed in the thesis (Chapters 3 & 4) which explored fruit and vegetables (frequency and quantity) as independent predictors of psychological health. This addressed the second aim to investigate the complex relationship between nutrient-rich FVI, nutrient-poor sweet and savoury snacks (and overall diet quality) and psychological health, within the general and young adult populations, while accounting for confounding factors. Overall, FVI predicted positive psychological wellbeing and symptoms of depression cross-sectionally, with frequency of fruit consumption being the most consistent across studies (study 1 & 2). Prospectively over four months (study 3), more frequent fruit consumption predicted higher positive psychological wellbeing and lower stress, while more frequent vegetable consumption predicted reduced symptoms of depression, highlighting that the frequency of consumption is important. Furthermore, frequent sweet and savoury snacking directly predicted a range of aspects of psychological health, including higher stress, anxiety, and lower psychological wellbeing (cross-sectionally), but the results were inconsistent across studies.

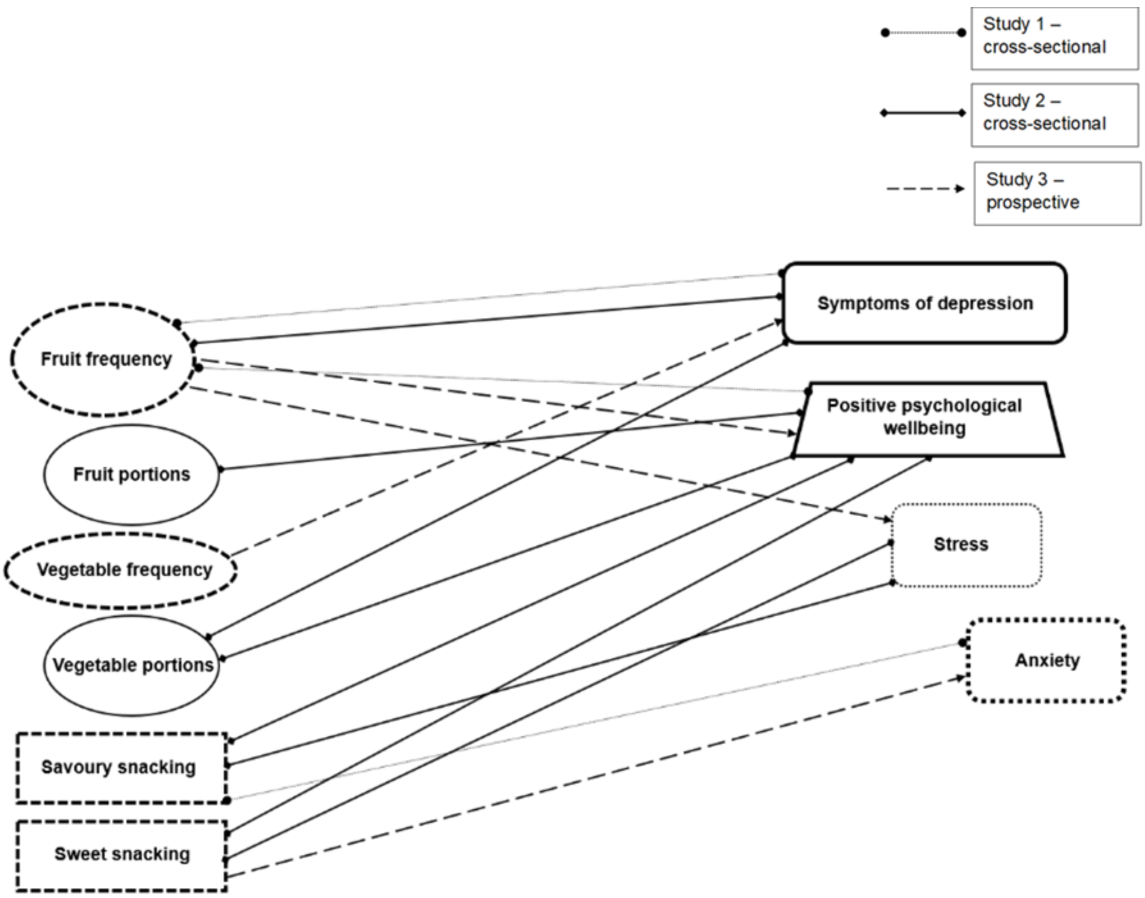
The third aim of the thesis was to evaluate whether cognitive processes act as a mediator in this relationship on psychological health. Consistent, cross-sectional mediation of cognitive failures in the relationship between savoury snacking and psychological health was found for all aspects of psychological health (study 1 and 2). Prospectively, cognitive failures only mediated the relationship between overall diet quality and stress (study 3), and no direct relationships were observed between overall diet quality and psychological health. The final aim of the thesis was to experimentally explore the effect of folate in supplement drink and food (vegetable) form, on mood, affect, cognition, and psychological health in healthy adults. Preliminary findings (Chapters 5 & 6) suggest that bioavailable folate consumption via a L-methylfolate supplement drink may affect perceptions of psychological health (anxiety and affect), with consistent reductions to negative affect (pilot study 1 and RCT). Some findings indicated an increase in positive mood and affect, such as content and positive affect (pilot study 1), happy and content mood (RCT exploratory results). No significant effects on cognition were seen across these experiments. Overall, precise acute effects of L-methylfolate were found for the reduction of negative emotional states (pilot 1 and RCT), and no effects were seen for a vegetable-based meal (RCT). This chapter summarises the findings presented in this thesis, which will be discussed in relation to previous research. The implications, then strengths and limitations of these findings will be presented, with recommendations for future research.

7.2 Overview of the thesis findings

7.2.1 Overview of the relationships between dietary intake and psychological health

The following Figure 7.1 summarises the significant, direct relationships observed for dietary intake and psychological health. The dietary predictors include fruit and vegetable consumption (frequency and portions), and savoury and sweet snacking. The psychological health outcomes include symptoms of depression, positive psychological wellbeing, stress, and anxiety.

Figure 7.1 The direct relationships found within studies 1-3, Chapters 3-4 of the thesis.



Note: Fruit or vegetables (frequency and portions) negatively predicted symptoms of depression and stress, but positively predicted positive psychological wellbeing. Frequent savoury snacking negatively predicted positive psychological wellbeing, but positively predicted stress and anxiety. Frequent sweet snacking negatively predicted positive psychological wellbeing, but positively predicted stress. Frequent sweet snacking negatively predicted anxiety.

7.2.2 Fruit and vegetables as predictors of psychological health

Findings of the systematic review

Previous research suggests there is a link between F&V consumption and psychological health, such as reduced symptoms of depression (Dharmayani et al., 2021; McMartin et al., 2013; Nguyen et al., 2017; Ribeiro et al., 2017) and increased positive psychological wellbeing (Blanchflower et al., 2013; Hong & Peltzer, 2017; Lesani et al., 2016; Mujcic & Oswald, 2016; Peltzer & Pengpid, 2017). Chapter 2 of this thesis evaluated whether F&V are equally associated with, and exert effects on, mental health and psychological wellbeing from the prospective evidence available (Tuck et al., 2019). Previous systematic review findings identified that F&V consumption independently protect against depression (Saghafian et al., 2018), but a superior influence of vegetable intake on psychological health (vs. fruit) has been reported (Molendijk et al., 2018). Broadly, the synthesis of the research identified that future research is required to understand the influence of fruit and vegetable intake (separately) on mental health and psychological wellbeing in healthy adult populations. This is supported by further systematic review findings in adults, which also suggest that fruit and vegetables are positively associated with psychological health (Glabska et al., 2020). However, very few studies (n= 3) had measured F&V separately in our systematic review and this is still the case (Glabska et al., 2020), which is surprising considering how different fruit and vegetables are in terms of their nutritional profiles (LaChance & Ramsey, 2018).

Overall, the systematic review findings supported a positive influence of F&V consumption on psychological health, and that regularly consuming close to recommended or higher levels (7-8 portions a day) of F&V may enhance daily psychological health through subjective psychological wellbeing. For positive psychological wellbeing, the results were more consistent for vegetable consumption, and stronger, more robust associations for vegetable compared with fruit consumption, for both men and women, were reported. Of the three studies that explored fruit and vegetable intake independently, two explored this in relation to psychological wellbeing and both reported that vegetable consumption increased psychological wellbeing (Conner et al., 2015; White et al., 2013). Furthermore, increased vegetable consumption (independent from fruit or, combined with fruit) was found to have enhancing effects on positive states of psychological wellbeing after brief periods of time (13 to 21 days) and over longer terms (six or twelve weeks to 2 years). When combined, even small increases in F&V consumption were associated with enhanced psychological wellbeing. Both F&V were positively associated with increased positive affect; whereas only the consumption of vegetables was associated with lower negative affect (i.e., this was not the case for fruit consumption). Thus, it was concluded that a preferential effect of vegetable (compared to fruit) consumption on psychological health, may exist.

Fruit and vegetable findings of the cross-sectional and prospective studies

Across Chapters 3 and 4, three studies explored the relationship between fruit and vegetable consumption (separately) and psychological health. The quantity (daily portions) and frequency of consumption was also evaluated separately, but when considering F&V intake more broadly, the collective results from two cross-sectional studies and one prospective study suggest fruit more consistently predicts psychological health. Fruit consumption predicted reduced symptoms of depression (specifically frequency across study 1 & 2), enhanced positive psychological wellbeing (across all three studies), and reduced stress (study 3). Notably, the first cross-sectional study did not identify any significant relationships for vegetable consumption. Therefore, the findings from study 1 did not support the findings from the initial systematic review which concluded that a preferential effect of vegetable consumption on psychological health may exist. On the other hand, the results from study 2 did find two significant relationships between vegetable portions and psychological health, with greater portions of vegetables predicting reduced symptoms of depression and higher positive psychological wellbeing. Importantly, the systematic review involved a synthesis of only prospective research, and when considering the prospective study 3 findings, these did show that higher baseline vegetable consumption (frequency) predicted reduced symptoms of depression after four months. However, none of the studies included in the systematic review measured the frequency of vegetable consumption, which may explain why eating the recommended *amount* of vegetables per day failed to prospectively predict the incidence of depression symptoms six years later, as highlighted by a study in the systematic review (Mihrshahi et al., 2015). The need to evaluate quantity and frequency of F&V consumption may explain the inconsistent findings in the systematic review for mental health. The F&V-psychological health relationships may differ when considered at one point in time, compared to over time because daily F&V portions may be more relevant to cross-sectional associations, whereas the impact of frequency (how often) may accumulate prospectively on symptoms of poor mental health. Additionally, frequency has been found to contribute more than daily portions to the variance in intake of most foods

(Thompson & Subar, 2017), which may explain why only prospective associations for frequency of consumption emerged.

As this thesis isolated the relationships for fruit and vegetables with psychological health, it is possible to compare the strength of the effects to identify if either represents a more potent target. When comparing the magnitude of effects across studies 1-3 for fruit and vegetables, the standardised beta values were very similar in size across different psychological health outcomes and studies. This was also the case for the independent variance explained, so this does not support the findings of the systematic review that vegetable consumption may be a preferential target.

Overall, the findings support previous literature that higher FVI is predictive of better psychological health (Glabska et al., 2020; Rooney et al., 2013), which includes both mental health and psychological wellbeing. More specifically, the findings support research that suggests F&V are independently and uniquely associated with symptoms of depression (El Ansari et al., 2014; Saghafian et al., 2018) and positive psychological wellbeing (Mujcic & Oswald, 2016; Ocean et al., 2019). Previous research has raised methodological issues as the reason for conflicting results, for instance the need for covarying for baseline general health status and mental health was highlighted (Brookie, 2018; Mujcic & Oswald, 2016). Study 3 covaried for baseline symptoms of mental health, which may explain the lack of prospective relationships observed, compared to previous literature (Winpenny et al., 2018). In addition, the research in this thesis investigated frequency and quantity of FVI (separately), and elements of psychological health within a single study each time to allow for within-study comparisons.

Vegetable consumption (daily portions) predicted positive psychological wellbeing once (study 2), but vegetable intake (portions and frequency) was more consistently predictive of symptoms of depression cross-sectionally and prospectively (study 2 & 3) than psychological wellbeing (study 2). The quantity of fruit consumed (daily portions) only predicted positive psychological wellbeing once (study 2), whereas the frequency of fruit consumption predicted higher positive psychological health within study 1 and study 3 (cross-sectionally and prospectively). However, the relationship between fruit frequency and positive psychological wellbeing seen in study 1 was not replicated in study 2. The reason for this could be that the UK-wide adult sample (study 1) consumed fruit more frequently than the young adult student sample (study 2), whereby within the second study this was 2-3 vs. 4-6 times a week in study 1. Furthermore, the second study was a smaller sample size (by 12%), and the simple regression p value was verging on significance ($p = .068$), which may explain why the relationship was not detected here. Indeed, previous prospective literature has identified frequent consumption of fruit as predictive of increased positive psychological wellbeing. For instance, frequent consumption of fruit snacks over a two-week period increases positive psychological wellbeing (Conner et al., 2017). Additionally, over thirteen days, F&V intake (separately) predict daily positive psychological wellbeing (Conner et al., 2015). Moreover, research shows that daily consumption of fruit predicts higher positive affect (Warner et al., 2017; White et al., 2013). Conner and colleagues (2015) also found that consumption of vegetables portions (but not fruit) was associated with reduced within-day negative affect in university students, however, this does not align with the acute vegetable-meal RCT findings in Chapter 6 (but there may have been inadequate power to detect this). Similarly, increasing vegetable consumption to meet dietary guidelines over the course of eight weeks has been shown to promote positive psychological wellbeing (De Leon et al., 2022). Additionally, the frequency of fruit consumption only predicted symptoms of depression cross-sectionally (study 1 & 2), which may mean frequent vegetable consumption over time is more important than fruit, for preventing depression.

The relationship observed between higher quantity and frequent consumption of vegetables and reduced symptoms of depression is supported by previous research that reports a lower odds or risk of depression (LaChance & Ramsey, 2015; Meyer et al., 2013; Saghafian et al., 2018), and fewer symptoms (Tsai et al., 2012). Over time the focus on frequency (how often) instead of daily portions may be required to sustain positive outcomes associated with habitual consumption. Collectively, the findings support this notion as six significant relationships were observed for frequency, but only three for portions of FVI. Thus, the findings across this thesis suggest that how often we consume F&V may be more important than the total amount we consume, which was previously suggested by Ocean and colleagues (2019) in their UK prospective study comparing FVI (frequency and quantity). The current findings also support a systematic review which reported that the more often fruit and vegetables are eaten by participants during a typical week (i.e., frequency of consumption), the better their psychological wellbeing is likely to be (Dharmayani et al., 2021). Within the current evidence base, F&V portions (quantity) has received more attention than the frequency with which fruits and vegetables are consumed (Dharmayani et al., 2021, 2022), which highlights the need for more research that evaluates both quantity and frequency of F&V in order for further comparisons to be made.

Prospectively, only the frequency of fruit or vegetable consumption was predictive of psychological health (fruit-stress, fruit-wellbeing, vegetables-depression). This builds on previous significant results from micro-longitudinal studies (14-21 days duration) which were identified through the systematic review in Chapter 2. However, these previous studies explored day-to-day fluctuations in psychological wellbeing as a result of daily portions or involved the provision of F&V. Prospective study 3 only observed a significant relationship over four months (not one month), which adds to the research in terms of identifying the timeframe by which relationships naturally emerge for how often F&V is consumed, compared to direct provision of F&V or analysis of within-day effects. Overall, the results imply that psychological health gains (reductions in symptoms of depression, stress, and increased psychological wellbeing) can occur after several months, and differential relationships emerge for F&V. The results emphasise that how often (e.g., how many times across a week) rather than how much (quantity) F&V is consumed may be more likely to promote improvements over time, as previously suggested by Ocean and colleagues, (2019). Additionally, FVI may need to be maintained in higher quantities to observe protective relationships for daily portions. Indeed, average FVI in the prospective sample were below the recommended levels (combined mean for F&V portions across all timepoints = 3.8; T1=3.7, T2=3.8, T3=3.9). Population level FVI has remained consistently below the recommended levels (combined average intake of 192grams per day of the 400grams recommendation), so there are margins for change (Woodside et al., 2023). Further, baseline dietary behaviour may vary between populations, thus having an impact on the relationships observed, and the precise dietary targets for improving both diet and psychological health may also differ (Crichton et al., 2013; Solomou et al., 2023).

Reflecting further on the reason for the differences between frequency and quantity, this may be because the frequency of consumption represents the habit of consuming nutrient-rich F&V on a regular basis, which in turn is likely to maintain micronutrient levels such as antioxidants or folate levels, which have relevant mechanistic action for psychological health (Bottiglieri, 2005; Martone, 2016; Moylan et al., 2014). It is reported that antioxidant intake is lower in adults with depression, which includes folate, polyphenols, and flavonoids (Huang et al., 2019). The precise prospective relationships identified that more frequent vegetable consumption (not quantity) reduced symptoms of depression, while more frequent fruit consumption increases psychological wellbeing and decreases perceived stress. Furthermore, across studies, fruit was more consistently related to positive psychological wellbeing, whereas vegetable intake was more consistently related to symptoms of depression. Thus, the differential effects of fruit and vegetables, could be due to different cumulative micronutrient consumption and action. Fruits are rich in antioxidants which are suggested to have a boosting impact on positive psychological wellbeing via the reduction of oxidative stress and free radicals (Rooney et al., 2013), which may more readily impact milder issues, such as stress (Radavelli-Bagatini et al., 2021). On the other hand, vegetables are known to have higher anti-depressive properties than fruit (LaChance & Ramsey, 2018), through higher levels of folate, that may more readily act on negative emotional states (Bottiglieri, 2000; Bottiglieri, 2005; Woo et al., 2006).

For other aspects of mental health, only frequent fruit intake was predictive of reduced stress over four months. No relationships were found for anxiety with regard to FVI, which may be explained by a recent metanalysis which showed a 'floor effect,' whereby healthy samples report low levels of anxiety, so it is difficult to observe associations with dietary intake (Firth et al., 2019). To evaluate this, further studies are required that recruit participants that exhibit sub-clinical levels of anxiety.

No significant relationships emerged for the relationship between FVI and cognition. This is supported by cross-sectional studies that also found no relationship between the adherence to nutrient-rich dietary patterns involving high FVI and cognition (Crichton et al., 2013). However, previous research has highlighted that FVI is associated with significantly improved cognitive performance (Lamport et al., 2014; Polidori et al., 2009), but none of the previous research involved young adults; all were over the age of 45 years, with the majority being classified as older adults. Thus, greater margins for change may be required to observe a positive relationship between FVI and cognition. Also, the current research predominantly focussed on cognitive errors (cognitive failures), so different measures of cognition may be required to detect an enhancement in cognition.

7.2.3 Snacking as a predictor of psychological health

Sweet and savoury snacking findings of the cross-sectional and prospective studies

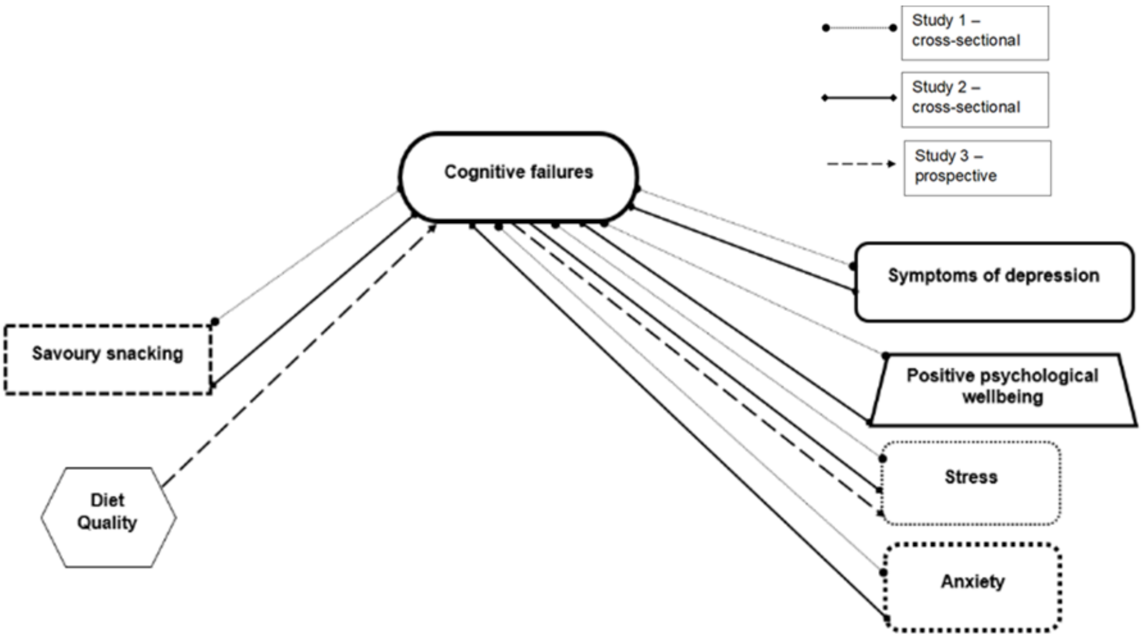
Across Chapters 3 and 4, three studies explored the relationship between sweet and savoury snacking (separately) and psychological health. Collectively, direct relationships between sweet and savoury snacking and positive psychological wellbeing, stress, and anxiety were found, whereas no significant links with symptoms of depression were observed. However, across studies the results were mixed. For study 1, a cross-sectional link between frequent savoury snacking and increased anxiety was found. Then, within study 2, associations between frequent sweet and savoury snacking, reduced psychological wellbeing and increased stress were found. Previous cross-sectional research has shown that nutrient-poor food consumption (e.g., snacks and biscuits high in saturated fat and sugar) predict mental health (Jacka et al., 2013). Dietary intake and quality (quantified as a dietary inflammation score) has also been significantly and positively associated with perceived stress scores, after adjusting for a range of health and demographic covariates (Knight et al., 2022). Additionally, snacking on chocolate or crisps in the afternoon for ten days resulted in increased anxiety (Smith & Rogers, 2014). Unexpectedly, study 3 found an inverse prospective relationship whereby higher frequency of sweet snacking at baseline reduced anxiety one month later, but this relationship did not persist at time point 2 (four months). The direction of the relationship implies that frequently snacking on sweet foods improves anxiety scores, which may be explained by comfort eating during stressful time periods in this student sample (Yannakoulia et al., 2008; Konttinen et al., 2010). Sweet snacking has been identified as a coping mechanism for lowering anxiety in young adults (Caso et al., 2020). Nevertheless, average TFEQ scores for this sample did not exhibit high levels of emotional eating. Hence, the current findings along with previous literature suggest that overall frequent consumption of nutrient-poor snacks may predict reduced psychological wellbeing and increased stress, with mixed results for anxiety.

Savoury (not sweet) snacking consistently predicted increased cognitive failures, and in turn psychological health across studies 1 and 2 (with no significant findings for prospective study 3). This is supported by previous studies which have found associations between dietary patterns of nutrient-poor snacking (both sweet and savoury) and cognitive failures (Chaplin & Smith, 2011; Crichton et al., 2013). Furthermore, research has identified that consumption of foods high in saturated fat or sugar e.g., crisps and biscuits (as measured by the savoury snacking item in our research), is detrimental to cognitive performance (Francis & Stevenson, 2011; Stevenson et al., 2020).The current findings provide support for previous literature that nutrient-poor food intake is associated with poorer hippocampal-dependent memory performance (Brannigan et al., 2015; Francis & Stevenson, 2011; Gibson et al., 2013). Higher self-reported intake of processed food has been associated with poorer memory (e.g., recall errors, memory retention) (Taylor et al., 2021). However, the current results suggest this is limited to savoury snacking, which could be due to the saturated fat content (Eskelinen et al., 2008; Morris et al., 2004, 2006). No prospective relationship was found over several months, but studies in older adults have found a link between nutrient-poor food consumption and cognitive decline over longer durations (Adan et al., 2019).

7.2.4 Cognition as a mediator

This next section will discuss the findings for cognition as a mediator. The following Figure 7.2 summarises the significant, indirect relationships found for dietary intake and psychological health.

Figure 7.2 The indirect relationships found within studies 1-3, Chapters 3-4 of the thesis.



Note: Frequent savoury snacking increased cognitive failures. Higher cognitive failures increased symptoms of depression, stress, and anxiety. Higher cognitive failures decreased positive psychological wellbeing. Higher diet quality decreased cognitive failures, and improved ratings of psychological health.

Cognitive failures mediate the relationship between savoury snacking and psychological health (cross-sectionally)

Across two cross-sectional studies (studies 1 and 2), an indirect relationship was found whereby cognitive failures mediated the relationship between savoury snacking and psychological health. This mediation occurred for all aspects of psychological health measured: symptoms of depression, anxiety, stress, and positive psychological wellbeing. However, no mediation was found prospectively for the relationship between savoury snacking and psychological health, nor sweet snacking. The psychological mechanisms by which diet affects psychological health have received very little attention, but cognitive processes have been hypothesised as a mediator (Rao et al., 2008). The current findings support the notion that cognitive processes may play a role in the link between dietary intake and psychological health, but this may be specific to memory-related errors, as no significant results were found for inhibitory control in study 1.

Cognitive failures are indicative of poorer cognitive functioning (Broadbent et al., 1982), and may be sensitive enough to detect early signs of deterioration in memory during daily life activities, which are associated with dietary intake. The consistent mediations observed extend previous research, as they highlight nutrient-poor, savoury snacking as a specific dietary component by which an increased vulnerability to cognitive, and consequently, mental health difficulties may occur. Previous research has identified nutrient-poor food consumption as relevant to memory function (Jacka et al., 2015; Taylor et al., 2021). Furthermore, experimental evidence shows that frequent consumption of high fat/high sugar intake has detrimental effects on cognition (Attuquayefio et al., 2017; Reichelt & Rank, 2017; Stevenson et al., 2020), and processed food consumption is associated with poorer hippocampal-dependent memory performance (Brannigan et al., 2015; Francis & Stevenson, 2011; Gibson et al., 2013).

Cognitive processes may be a psychological mechanism by which dietary intake influences psychological health. This is supported by the literature showing associations between impaired cognition and poor psychological health (Austin et al., 2001; Knight et al., 2020). General cognitive status has been associated with mental health (Dotson et al., 2008) and psychological wellbeing (Gates et al., 2014). Overall, the mediation results support previous findings that snacking on nutrient-poor processed foods, such as crisps, is associated with increased cognitive and psychological problems (Sangsefidi et al., 2020; Smith & Rogers, 2014) and builds on findings that reported a positive relationship between frequent unhealthy snacking and higher cognitive failures and stress (Chaplin, & Smith, 2011). Moreover, inflammation and oxidative stress are known pathways related to a pro-inflammatory diet involving high intake of saturated and trans-fatty acids found in snacks, with low intake of antioxidants from fruits and vegetables (Giugliano et al., 2006). This inflammation can cause progressive cognitive decline and hinder the biological pathways that regulate cognition, stress, and emotion (Byers & Yaffe, 2011; Franceschi et al., 2018; Wärnberg et al., 2009). Indeed, higher levels of inflammation (proinflammatory cytokines) and oxidative stress are seen in depressed individuals, hence the inflammatory theory may explain the development of poor psychological health through dietary intake (Grajek et al., 2022). It is plausible that savoury snacking may have a negative effect on psychological health, via an increase in cognitive failures. This may be explained by how nutrient-poor foods have detrimental effects on cardio-metabolic health which is important for healthy brain function (Adan et al., 2019). Moreover, frequent savoury snacking may have consequences for brain health due to their energy-density and calorific content; i.e., other essential nutrients or antioxidants from nutrient-rich foods that support biological processes and are required for optimal brain function, may be displaced, or not regularly consumed as an adverse consequence of frequent snacking (Young et al., 2020).

Cognitive failures mediate the relationship between diet quality and stress (prospectively)

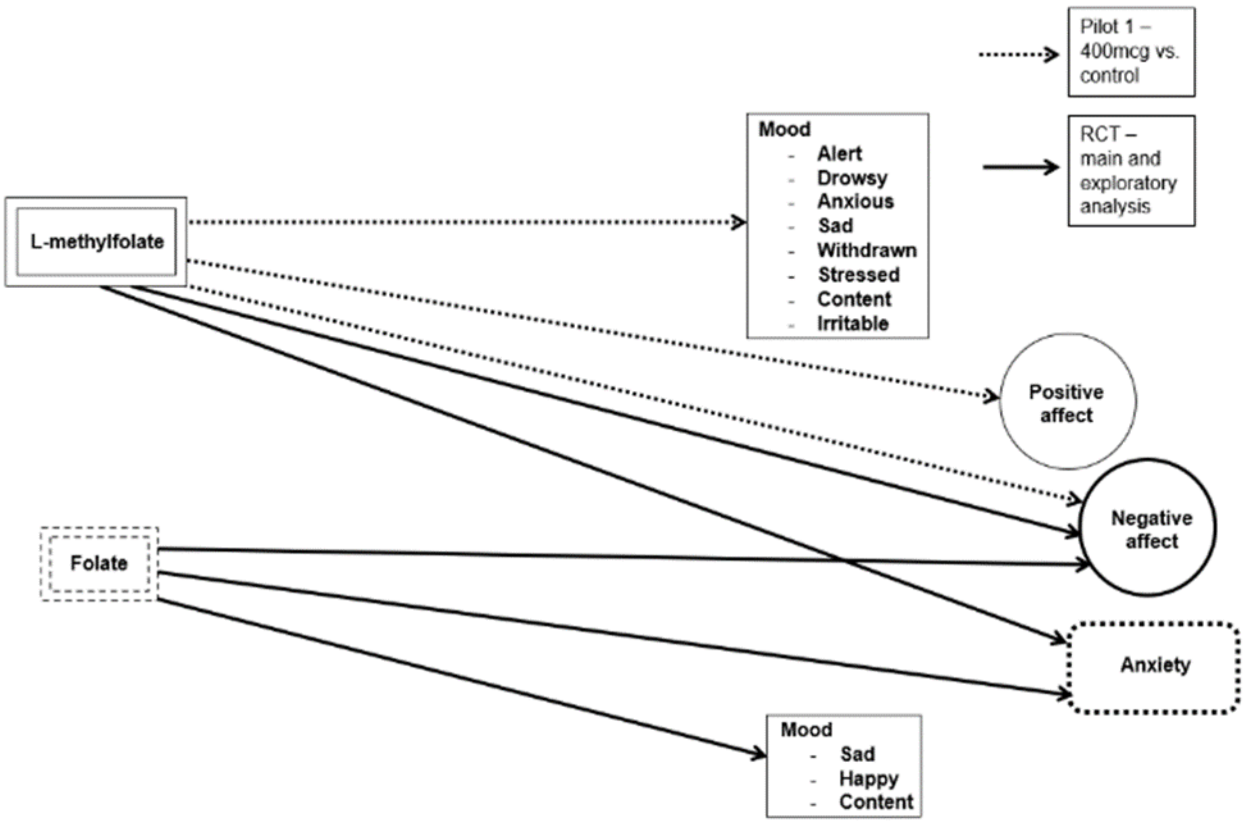
Study 1 of this thesis (Chapter 3) observed significant relationships between frequency of fruit consumption, symptoms of depression and positive psychological wellbeing (Tuck et al., 2022). Then, overall diet quality was explored in addition to the potential impact of FVI (separately) on psychological health in the second and third studies, however, no significant direct relationships were observed across both studies (cross-sectional or prospectively) for diet quality. Indeed, there is previous research that has also not observed a prospective relationship between overall diet quality and depression, after adjustments for covariates (Chocano-Bedoya et al., 2013). On the other hand, there are many studies to suggest that diet quality is associated with psychological health. For instance, better quality diets are linked to fewer symptoms of depression, and improvements in emotional functioning (Jacka et al., 2010, 2011, 2013). However, the direct relationship between overall diet quality and psychological health may rely on the use of interventions to increase overall diet quality over time.

Interestingly, one indirect prospective relationship was found in study 2 (Chapter 4); as overall diet quality increased, this reduced cognitive failures, and lower cognitive failures predicted lower stress scores. This indirect relationship was only observed after four months, not one month. Previous research suggests that better diet quality predicts fewer cognitive errors (Ye et al., 2013), however, few studies have explored the relationship between overall diet quality, alongside food groups and cognition, thus this research has expanded the previous literature. The indirect result found in study 3 could represent a specific pathway by which diet quality could protect psychological health. Increased cognitive failures are known to increase stress (Chaplin, & Smith, 2011), so reducing impaired cognition, such as everyday memory errors, through improving overall diet quality may be a promising preventative avenue. No further significant relationships for diet quality were found across the studies, however, it is important to consider that on average, the samples had low overall diet quality scores. The majority of scores could not be classified as high quality (following a healthy dietary pattern requires a score above 12; for study 2 and across all timepoints during study 3 all means were <10), which may explain the wider null relationships observed for diet quality scores and psychological health, as scores fell short of the UK dietary recommendations, and thus, it may have been unlikely that an enhancing effect could be detected.

7.2.5 Findings of the folate experiments

This next section will discuss the experimental results for L-methylfolate and folate on mood, affect, cognition and psychological health across two pilot studies and one RCT (Chapters 5 and 6). These fulfil the final aim of the thesis, which was to experimentally explore the effect of folate (in supplement and food form) on mood, affect, cognition, and psychological health in healthy adults in controlled laboratory settings. The following Figure 7.3 summarises the significant, experimental effects found in studies 4-6 of the thesis.

Figure 7.3 The experimental effects found within studies 4-6, Chapters 5-6 of the thesis.



Note: L-methylfolate increased positive affect, alert, and content mood. L-methylfolate decreased negative affect, anxiety, drowsy, anxious, sad, withdrawn, stressed, and irritable mood. Folate consumption decreased negative affect, anxiety, and sad mood. Folate consumption increased happy and content mood. No significant effects were found in pilot experiment 2.

Across two experimental studies (pilot 1 and the RCT), initial proof-of-concept was established for L-methylfolate supplementation via a drink, on perceptions of psychological health in the past week, including increased positive affect, reduced negative affect and anxiety. Both studies found significant effects on the reductions of negative affect, but only the RCT found an effect on anxiety using the DASS-21, even though the same measure was used. This could be due to the larger sample size of the RCT compared to pilot study 1. Pilot study 1 found an additional significant enhancing effect on positive affect, whereas the RCT did not replicate this. Therefore, the effects may be more pronounced on negative emotional states because the RCT used 200mcg (to enable the mcg to match a vegetable-based meal), whereas pilot 1 used 400mcg. This suggests that to identify an effect on positive affect or content mood, a higher dose of L-methylfolate may be required. Furthermore, in pilot study 1, significant effects on facets of positive and negative mood during the experiment were found including ratings for: alert, drowsy, anxious, sad, withdrawn, stressed, content and irritable mood. Again, fewer positive facets of mood were influenced (content and alert) compared to negative (drowsy, anxious, sad, withdrawn, stressed, irritable), but alertness and drowsiness may represent arousal. In total, six VAS items measured negative mood states (all showed effects) and five measured positive mood states (two effects seen). No effects on mood were found in the pilot study 2 or the RCT (excluding the maximum folate versus no folate consumption exploratory analysis). In pilot study 2, no significant main effects of condition were observed, across any of the key measures (mood, affect, cognition or perceptions of psychological health). In the RCT, no significant effects of the meal or interaction with the L-methylfolate drink were seen for affect, mood, cognition, or psychological health. No further effects of L-methylfolate drink were observed for perceptions of psychological health (depression, anxiety, or stress scores), psychological wellbeing, or other positive mood states across the studies. Moreover, no significant effects were observed across the three experiments for cognition (cognitive failures or the delayed-word recall task).

Follow up exploratory analysis conducted for the RCT compared the difference between the control (rice-meal with plain water, no folate consumption) with the maximum folate consumption (vegetable-rice, folate-rich meal with L-methylfolate drink, maximum folate consumption of 400mcg in total). This showed there was an effect of L-methyfolate+folate-rich meal to reduce negative affect and perceived anxiety in the past week, compared to those who did not consume any L-methyfolate or food folate, while including general health rating, household income, age, and leafy green vegetable intake (daily portions) as covariates. However, the effect of condition on anxiety scores was specific to the DASS-21 subscale (no significant effect was found for the HADS or VAS anxious mood). For mood, higher happy, content, and lower sad mood across the time course of the experiment were seen for the active (maximum folate consumption) condition compared to the control (no folate consumption). No further significant effects on mood, affect, cognition or psychological health were observed.

The selective L-methylfolate drink effects on the reduction of perceptions of negative emotional states (perceived anxiety and negative affect in the past week) are supported by previous research. For instance, Yary (2013) found that folate intake was associated with significantly lower depressive affect, but not positive affect, in university students. Furthermore, RCT findings show that one-hour after the consumption of one multi-vitamin tablet containing 500mcg of folic acid, benefits to anxiety DASS-21 scores were found, but the VAS anxiety ratings also failed to reach statistical significance (Macpherson et al., 2015). Additionally, decreased stress and increased calmness were also observed after the single dose. Further research has also shown that as red blood cell folate concentrations increase, negative affect variability decreases in healthy males (Williams et al., 2008). This may explain the consistent effects observed for negative affect across pilot study 1, the RCT main and exploratory analysis. Although, the results from Williams and colleagues (2008) are related to longer-term consumption of folate, the current results build on previous associative or observations of dietary intake of folate to suggest that acute L-methylfolate manipulation may have a fast-acting effect on negative affect. The current use of bioavailable L-methylfolate is likely to have been metabolised and circulating in the body (between 28-42 minutes), which aligns with the timeframe for approaching peak absorption (Finglas et al., 2003). As the covert L-methylfolate drink manipulation cannot be explained by psychological expectancies, a biological explanation for the acute effects seen is likely.

Across experiments 4-6, no acute L-methylfolate effects were found on other aspects of psychological health including symptoms of depression or stress, and positive psychological wellbeing. A previous systematic review of B vitamin supplementation research (including folate) in healthy adults on several aspects of psychological health showed that 5/8 studies found benefits to mood, but the effect for symptoms of depression did not reach statistical significance (Young et al., 2019). The significant effects on mood found such as sad, stress, and withdrawn (pilot 1) and sad mood (RCT) may put individuals at risk of symptoms of depression over time, as low mood and affect are interrelated and may contribute to symptoms of depression (Al Ammar et al., 2020). However, mood, affect and anxiety may be more sensitive to L-methylfolate supplementation compared to symptoms of poor psychological health because mood and affect are more momentary (transient), whereas symptoms of depression represent chronic mood states. The current findings add to the acute fruit flavonoid experimental research showing effects on positive affect (Khalid et al., 2017), by showing that folate may also impact perceived affect. More specifically, the blueberry flavonoid drink (via a fruit-based micronutrient) only increased positive affect, no effect was seen for negative affect. The current research suggests that a L-methylfolate drink (a predominantly leafy green vegetable-derived micronutrient) may also exert a precise (opposite) effect on negative affect (as both the previous and current research used the PANAS measure of affect).

With regard to the pilot study null results for mood and affect, the absence of mood-effects seen in pilot study 2 may be due to a lack of power to detect subtle differences between the three conditions (0mcg, 200mcg, and 400mcg of L-methylfolate). Alternatively, a genetic mutation (the MTHFR gene or C677T folate polymorphism) can impair folate metabolism and absorption (Gilbody et al., 2007b). This is found in the human genome of between 8 to 20 percent of individuals, depending on ethnic and geographic variation (Gilbody et al., 2007b). This could explain the null effects found in pilot experiment 2, if the sample had recruited a larger proportion of individuals with this gene, compared to the pilot experiment 1 and the RCT.

Regarding the cognitive results, only significant effects of 400mcg of L-methylfolate on increased alertness, and decreased drowsiness in the first pilot study were found. These may represent more readily detectable fluctuations in general cognitive arousal. Acute improvements to alertness and reduced drowsiness have been previously found for supplementation with 400mcg of folic acid (Kennedy et al., 2010, 2011, 2016). Multi-vitamin supplementation in healthy older men also showed improvements in alertness and day-to-day functioning (Harris et al., 2011), so folate may offer similar effects. Increased physiological arousal (alertness) after the consumption of bioavailable folate may in turn influence higher order cognitive functions, and consequently psychological health. No significant effects of folate (via the vegetable-based meal or bioavailable supplement drink) on wider cognition (cognitive failures or word recall) across the experiments were found. This does not provide support for previous research across 20 studies that found FVI is associated with significantly improved cognitive performance (Lamport et al., 2014), however, none of the previous research explored the acute effects of vegetables or L-methylfolate. Improvements to nutrient status over time may be required to influence cognition, as previous research using a battery of cognitive tasks (known to be sensitive to nutritional status) did not observe any acute effects 1-2 hours after a single dose of a multi-nutrient supplement (including 500mcg of folic acid) (Macpherson et al., 2015).

No significant effects for the vegetable-based meal were found, however, as suggested by the systematic review in Chapter 2, large quantities of vegetables rather than the provision of one vegetable-based meal may be required to see effects on psychological health (Tuck et al., 2019). Indeed, no previous experiments have manipulated vegetable intake in this way for comparisons to be drawn. However, a sixty-day randomised trial manipulating green juice intake (containing green cabbage leaf, lettuce leaf, cucumber, and apple) compared to a placebo control, resulted in increased circulating folate levels, but no significant differences between groups for wellbeing or anxiety scores (Chiochetta et al., 2018). Thus, in an acute setting, micronutrient supplementation may be a more potent target and easier method for improving specific aspects of psychological health such as anxiety, compared to the consumption of vegetables in meal or juice form.

The findings imply that the covert consumption of highly bioavailable folate as a drink, rather than overtly in the form of a meal may have an acute, relatively immediate effect on reducing negative affect, anxiety, or negative mood states. L-methylfolate is the active and bioavailable form of folate; it is the only form of folate that is ready for the brain to synthesise because it immediately crosses the blood brain barrier (Stahl, 2008). This may explain why the effects were not found for the vegetable-based meal. L-methylfolate impacts the synthesis of neurotransmitters including serotonin, dopamine, norepinephrine, which all contribute to psychological function (Leahy, 2017; Muscaritoli, 2021). Also, L-methylfolate modulates the processing of homocysteine (a toxic amino acid) into methionine (a beneficial amino acid) (Bottiglieri, 1996). Methionine is a precursor of SAMe (S-adenosylmethionine) which also has anti-depressant properties due to its involvement in the metabolism of neurotransmitters relevant to mental health (Bottiglieri et al., 2000, 2005). This mechanism is known as the “homocysteine hypothesis,” which is another biological pathway by which dietary intake may impact psychological health (Folstein et al., 2007). Nutritional deficiencies in folate can result in elevated homocysteine levels, and consequently reduce the synthesis of neurotransmitters, which could impact transient and chronic mood (Beydoun et al., 2010; Kaplan et al., 2007; Rooney et al., 2013). Research has shown that when individuals with depression were given a daily 500mcg folic acid supplementation for ten weeks (alongside their usual medication), serum homocysteine status changed by over twenty percent (Coppen & Bailey, 2000). Research suggests that only prolonged supplementation with folate (weeks to years) may reduce the onset and severity of depressive symptoms in people with poor nutritional and/or psychological status (Almeida et al., 2015). Populations at risk of poor psychological health, such as young adults, may benefit from folate supplementation over time, but future work is required.

Collectively, the results from the three experimental studies suggest that folate may act as a potential mechanism for reducing negative affect, negative mood (e.g., sadness), and perceived anxiety, which could accumulate over time to influence symptoms of poor mental health (e.g., dysphoria or depression). The current results from studies 4-6 (in Chapters 5-6) should be interpreted with caution, as due to COVID-19 restrictions, the total sample sizes for the statistical power calculations were not met, especially in pilot experiment 2 exploring the dose-dependent relationship. However, across studies, there were consistent trends when evaluating the direction of the means. Consequently, there is a need for adequately powered experimental work to better understand the causal relationship between folate from food, or L-methylfolate supplement form, as a bioactive micronutrient found in vegetables with potential effects on psychological health. Overall, the results add to the limited evidence available for micronutrient supplementation in non-clinical populations (Jackson et al., 2021; Young et al., 2019). Notably, the acute effects observed were through modest folate levels aligned with RNI (400, 200 mcg), which suggest that folate in highly bioavailable form of folate could be consumed on top of eating F&V regularly to prevent negative aspects of psychological health. Likewise, as the experimental studies used a modest L-methylfolate microgram dose, this could be increased further, which may yield larger effects for the reduction of negative emotional states.

7.3 Strengths and Limitations

Strengths of the research include the range of methodologies used: a systematic review, online cross-sectional and prospective studies, and laboratory-based experimental studies, form the thesis. Furthermore, the research involved a thorough evaluation of dietary intake including nutrient-rich and nutrient-poor components, with overall dietary intake, and a range of aspects of psychological health were measured. By investigating different food groups and elements of psychological health within and across studies, valid comparisons were made. Furthermore, the replication of methodologies across a range of samples (UK general adult population, university students and young to middle aged adults) enabled the comparison of results and identification of consistent dietary predictors. Both the quantity and frequency of FVI was examined to further understanding of the links with psychological health with greater precision. In addition, a novel exploration of direct and indirect relationships through mediation was conducted.

Moreover, the experimental data involved manipulations of folate intake that have not been previously investigated. A common criticism of previous supplementation studies is that the doses are high and unachievable through food (Ohrvik & Witthoft, 2011). The current manipulation matched the supplement and food form of folate to the current RNI for folate, thus was more realistic and relevant to consumption in real life. Another strength of the experimental studies was the covert, undetectable manipulation of folate through the L-methylfolate drink, as response expectancies could not be at play, this suggests the role of biological processes. In addition, all of the studies in this thesis included lifestyle behaviours beyond dietary intake as covariates, which strengthens the confidence in the significant relationships found. By adding important confounding variables into the analysis (e.g., general health rating, exercise, sleep, alcohol intake etc.) the studies within this thesis consider dietary intake as a lifestyle behaviour that is embedded in a biopsychosocial framework (Rossa-Roccor et al., 2021).

Limitations of the research include that the associations observed (study 1 and 2) could have been confounded by how individuals who consume higher F&V may also maintain an overall healthy lifestyle, or be highly motivated when it comes to their health. For instance, people who eat more F&V, may rate their health more highly overall. Moreover, a health-focussed attitude may be accompanied by higher resilience or willpower, which in turn may protect psychological health (Rahe et al., 2014; Sanchez-Villegas et al., 2009). The inclusion of other lifestyle behaviours such as exercise as covariates in the analyses allowed the unique contribution of F&V to be examined, however, the use of self-reported minutes as an indirect measure may have lacked validity (Dinger et al., 2006), especially because other studies (particularly, more recent studies) have opted for the use of wrist-worn activity trackers instead (Sutliffe, 2018). Indeed, the reliance on self-report for dietary intake and lifestyle behaviours can be prone to reactivity bias, recall or social desirability bias (Kirkpatrick et al., 2018; Miller et al., 2008). However, this was deemed practical considering the time and cost restraints for collecting data on a large scale. Additionally, the relationship between dietary intake and psychological health may be due to reduced body mass index and associated inflammation (Oddy et al., 2018). BMI has been shown to have a non-linear association with depression, whereby those who are underweight or obese tend to have higher odds of depression, compared to individuals that are healthy weight or overweight (De Wit et al., 2009). BMI was calculated and included in the analyses, but again, when relying on self-report, this is prone to inaccuracies (Gorber et al., 2007). Hence, it is a strength of the thesis that the laboratory-based experimental studies did measure height and weight directly during the session, to allow for baseline differences between groups to be considered. Another limitation across studies was that the screening process for participants did not include assessment of vitamin or mineral supplement intake, which may have confounded the results. The main purpose of the screener was to recruit healthy adults, so this asked about medication use only.

Regarding the experimental evidence in Chapters 5-6, overall, the studies were underpowered due to COVID-19 restrictions halting data collection. Notably, a recent synthesis of dietary and nutrient-focussed interventions for psychological health found small effect sizes for most studies (O’Neill et al., 2022), therefore, much larger sample sizes may have been required to have confidence in the significant and non-significant effects found. The lack of folate effects via the vegetable-based (spinach) meal may be due to the degrading of folate in spinach during brief exposure to heat during the meal preparation process. Research has found that only raw vegetables/salad, but not cooked vegetables were linked to depressive mood states (Brookie et al., 2018). More specifically, spinach has high water content, which can cause the removal or leaching of folate during heating

(Czarnowska-Kujawska et al., 2022). Even though the minimum amount of cooking time was utilised to warm the spinach to create a spinach-based meal, this could have weakened the impact of the vegetable-based folate manipulation. Thus, future research could manipulate the consumption of raw folate-rich vegetables instead. Furthermore, the lack of complete blinding of the investigator to condition allocation during experimental studies 4-6 (Chapters 5 & 6) is also a methodological issue to note.

Another limitation within Chapters 5-6 (pilot studies and RCT) was that measures of perceptions of psychological health were only completed once as they focussed on subjective ratings in the past week or longer, meaning no pre-manipulation comparison could be made. However, baselines differences were assessed between groups and the VAS mood assessment acted as a within-person measure as this assessed mood in the present moment multiple times for each participant.

7.4 Implications and Future Directions

This thesis highlights that dietary intake appears to be linked to specific aspects of psychological health, both directly and indirectly, and previous research supports that the strongest lifestyle predictors of psychological wellbeing are exercise and habitual F&V consumption (Chilver et al., 2023). Changing an individual's whole diet is intensive, thus, focussing on specific dietary recommendations, such as minimal consumption of nutrient-poor, processed foods, and plentiful consumption of nutrient-rich foods, may be a more viable starting point (Rajaram et al., 2019). The findings from this thesis suggest that simple changes to dietary habits, such as increasing FVI frequency and quantity while reducing savoury snacking, may be one strategy to promote psychological health.

The frequency of F&V appears to be consistently linked to positive psychological wellbeing from the results in this thesis, thus, interventions that aim to increase how often F&V are consumed may result in wellbeing gains over time (Blanchflower et al., 2013; Ocean et al. 2019). Furthermore, consuming F&V more often may reduce consumption of nutrient-poor foods, such as chocolate or crisps (sweet and savoury snacking), via a "substitution effect" (Ocean et al., 2019). Recently, it has been advised that energy-dense snacks should be replaced with nutrient-rich F&V, as well as substituting higher-energy ingredients in meals with F&V to promote positive health outcomes (Centers for Disease Control and Prevention, 2022; Skoczek- Rubińska et al., 2021). Therefore, future research could involve interventions focussed on substitution effects (not simply addition) of replacing nutrient-poor snacks with nutrient-rich F&V (e.g., having an apple instead of a packet of crisps) as one potential intervention for improving psychological health. Studies could build on the snacking intervention research by Smith and Rogers, (2014). Previous data focussing on nutritional outcomes shows that as FVI increases, fibre, vitamin C and carotene increases, while fat intake decreases (Fulton et al., 2016; 2017). Therefore, changes to dietary habits such as switching nutrient-poor for nutrient-rich foods could enhance both nutrient status and psychological health.

A suboptimal diet is considered detrimental to healthy brain function and has been implicated in increased risk of mental health and memory issues (Davison et al., 2017; Livingston et al., 2017). The consistent, cross-sectional mediation of cognitive failures between savoury snacking and psychological health observed (Chapters 3 & 4) supports this, while overall diet quality may protect cognitive processes, and in turn reduce experiences of stress. Therefore, further experimental investigation of cognitive processes as mediators between savoury snacking and psychological health is required, particularly to confirm causality. Future work could also evaluate whether FVI and folate consumption impact cognition through the experimental measurement of other cognitive processes, using extensive computerised cognitive assessment involving highly demanding, effortful tasks, as these are suggested to be more sensitive to subtle nutritional effects (Bryan et al., 2022; White et al., 2015). This will build a greater understanding of the role of cognition between dietary intake and psychological health. Other psychological mechanisms could also be explored further, such as expectancy-related benefits associated with consuming F&V, that is, the placebo effect that consuming healthful food leads to increases in positive mood and perceptions of enhanced wellbeing (Smith et al., 2022). Smith and colleagues (2022) recently developed a new scale for assessing positive F&V expectancies, which collects information about participant beliefs without prompting what those beliefs might be. This scale was not available during the design of the studies within this thesis, however, could help to shed light on whether engaging in healthful eating habits alone can positively impact psychological wellbeing.

To understand the possible mechanistic pathways by which FVI influence psychological health, the investigation of biomarkers (i.e., the evaluation of biochemical changes) to identify how effects occur could be a part of future interventions. The micronutrients in F&V are hypothesised to indirectly impact brain health via their reduction in inflammation, improved antioxidant, or microbiome effects, that may work synergistically (Marx et al., 2018). Hence, future work could evaluate the antioxidant action of FVI using a Biophotonic scanner which assesses skin carotenoid, antioxidant levels (Braune et al., 2018). This is an objective non-invasive, convenient technique which has been recently shown to strongly correlate with blood circulating antioxidant levels (Henning et al., 2023). Relatedly, this measure could also be used as a validation method for self-reported FVI, with biomarker levels being used to establish a level of agreement. This could help inform the causal inferences for the underlying effects of dietary intake on psychological health.

Finally, the onset of psychological disorder commonly develops before the age of 25 years (Solmi et al., 2022), with approximately 30% of young adults reporting experience of a mental illness (Merlo & Vela., 2022). Future F&V intervention studies could recruit young adults with sub-clinical, low mood, or dysphoria to evaluate new preventative strategies for protecting psychological health. Evidence suggests that when baseline symptomology is considered, the magnitude of effects may become clinically meaningful, but this is yet to be thoroughly investigated (Appleton et al., 2023). Likewise, recruiting low F&V consumers may realise larger effects on psychological health (Blank et al., 2007). Indeed, young adulthood is a critical period for establishing health patterns, including diet, which will carry over into adulthood, and concerningly only 13% of 15–29-year-olds meet the recommended intake of F&V (5-a-day) (Ocean et al., 2019). Thus, increasing FVI to at least the recommended level may result in noticeable psychological wellbeing gains (Blanchflower et al., 2013; Glabska et al., 2020), and this in turn could help to prevent poor psychological health throughout the lifespan.

7.5 Conclusion

In conclusion, promoting dietary habits that lead to better psychological health, could improve individual wellbeing, alleviate strain on healthcare systems, and reduce the economic cost associated with both poor psychological health and cognitive failure (Adan et al., 2019). Focussing on prevention rather than treatment is important (Grajek et al., 2022), and the findings from this thesis suggest that higher and more frequent F&V consumption could boost psychological wellbeing in healthy adults and reduce symptoms of depression. Additionally, the indirect relationships seen in this thesis suggest nutrient-poor, savoury snacking may be a dietary habit to target for protecting cognition (reducing the likelihood of cognitive failures related to memory), which in turn could protect psychological health. Furthermore, the effects of L-methylfolate on reducing negative emotional states show promise as an adjunctive supplement to address nutritional insufficiency. These findings suggest modifiable lifestyle strategies for improving psychological health, which if implemented at the population level, could contribute to considerable public health benefits. Overall, this thesis provides a significant contribution to our understanding of the direct and indirect relationships between dietary intake, cognition, and psychological health. However, this is a novel area of research, which requires further work to elucidate the magnitude and unique contribution of dietary intake for promoting psychological health.

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Appendices

Appendix A: Word list for the delayed-recall task

Low frequency:	**High frequency:**
STABLE RUBBLE BEETLE CYMBAL LOTION	BUTTER WEAPON MUSCLE FLEECE PEPPER
Low imageability:	**High imageability:**
BRANCH POETRY FIDDLE GINGER RATTLE	ARTIST WINTER HURDLE TONGUE ORANGE

Appendix B: Meal Preparation for RCT

The meal preparation steps were as follows:

1. Use a measuring jug to add 1000 ml of tap water into a saucepan to boil (set hob heat setting to 6, takes 3 minutes to boil)
2. Weigh 30.4g uncooked rice
3. Rinse rice in colander with cold running water
4. Place rice in saucepan of boiling water
5. Cook rice for 15 minutes on hob heat setting 4 (time this on phone stopwatch)

If vegetable-based meal:

a. Place second saucepan on hob and set hob heat setting to 3 (low heat)
b. Add 8g of margarine to the warm saucepan
c. Weigh 137g of already washed spinach leaves from the bag and add to saucepan
d. Cook spinach on hob heat setting 3 for 3 minutes (time this on phone stopwatch)
e. Mix and stir together cooked rice with cooked spinach

6. Add and stir through salt seasoning *(also add the margarine if non-vegetable-based meal)*
7. Serve in a bowl (always use the same bowls, identical across conditions) with a fork, spoon, and serviette presented on a white tray

Appendix C: Rationale for piloting spinach as the vegetable for the meal manipulation

Why spinach for the vegetable-based meal? Natural food-based folate is found to be particularly high in fresh leafy spinach, at approximately 200mcg per 100g (238.45 ± 13.36mcg) (López-Nicolás et al., 2014). Spinach is a vegetable with high acceptability (overall liking, flavour, and texture liking) and popularity due to it being one of the less bitter vegetables (Batziakas et al., 2019). Furthermore, the folate in spinach has been found to have a high absolute absorption score similar to synthetic folic acid, demonstrating in theory similar bioavailability (Ohrvik & Witthoft, 2011). Additionally, an evaluation of the relative folate bioavailability of spinach, wheat germs, Camembert cheese, compared to a control acid as the reference dose, identified that the highest bioavailability is in spinach (Mönch et al., 2015).

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