Effect of supplementation of flavonoid-rich fruits on cognitive performance and mood in children aged 11-13 years old.

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Declaration

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

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Abstract

Fruits rich in flavonoids have shown positive effects on cognitive performance, mental health and mood in both animals and adult humans, possibly via their ability to influence neuronal connectivity and protect against neurodegeneration. However, evidence regarding the translation of such effects to children is more limited. The aim of my thesis was to evaluate whether supplementation of flavonoids from fruit can improve cognitive function, mood, and academic attainment in 11-13 year old children.

Initially, a 12 weeks daily supplementation study using 40 gram portion of high-flavonoid (HF) or low-flavonoid (LF) fruit with baseline and post-intervention measurement of cognition performance and mood was carried out. Improvement in a composite score of executive function and reduction in negative mood affect following HF intake was observed. However, no effect on memory and overall academic attainment were found.

To test and extent the reliability of the effect of flavonoid supplementation on executive function and negative affect seen in Experiment 1, a second supplementation study was conducted comprising of a double portions of HF and LF fruits (80 gram) compared to a control group over 7 week intervention. The means for executive function and negative affect revealed the same trend as in the previous experiment however, the significant differences were not replicated.

Finally, the discrepancies in results of Experiment 1 and 2 were further explored in a final experiment where children were supplemented with 80 gram of HF or LF fruit conditions for 12 weeks. Paper and pencil cognitive tasks and mood were measured at baseline and post intervention. Disappointingly, despite the much larger sample size, an absence of significant differences between groups was observed similar to Experiment 2.

In conclusion, mixed evidence at best was found for the effects of flavonoid rich fruit supplementation on composite score of executive function and mood, with no evidence of effects on academic performance in 11-13 year old children. Crucially, though this pattern was not consistently observed throughout the studies. Further research in children is required to investigate the association between flavonoid rich fruits in cognitive and academic outcomes utilising longer supplementation durations and higher content of flavonoids to better characterise their potential effects in school age children.

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Chapter 1 Introduction

1.1 Introduction

One of the greatest challenges that the world faces is the explosion in its population. The Royal Society reports that the world population increased from 2 billion people in 1930 to 6.8 billion in 2010. In addition, Black (2010) estimates that the world population could increase to a peak of 9 million people by 2050. Importantly, a report by the United Nation (UN) Population Division states that the expected number of individuals aged over 60 will be almost 1.9 billion in 2050 (United Nation, 2005). In Thailand, the Institute for Population and Social Research at Mahidol University reported that the Thai population aged 60 and older increased to 6 million in 2005 and may triple this by the year 2035 (http://www.ipsr.mahidol.ac.th/IPSR/AnnualConference/ConferenceII/Article/Download/Article02.pdf). It is clear that with an increasingly ageing population there is an increase in the incidence of age-related neurodegenerative disorders, and this will significantly affect the cost of health care around the world.,

Age-related cognitive decline has been linked to oxidative stress and inflammation, which ultimately can lead to neurodegenerative diseases such as Alzheimer disease, vascular dementia (Joseph, 2000) and Parkinson disease (Joseph, 2007). Exploring the benefits of foods and nutrients to protect against inflammation and neurodegeneration is a crucial step in maintaining cognitive health (Mangels, 2003; Casadesus G, Shukitt-Hale, 2002). It is widely known that diet is an important lifestyle factor mediating human health. Consumption of a healthy diet in early life may result in positive effects on life-long health. In particular, consuming high portions of fruits and vegetables throughout life has a significant effect on cognitive function (Kang, 2005; Mangels, 2003). Conversely, there is evidence that intake of foods high in saturated fats, over time, can lead to negative effects on cognition and increases in neurological dysfunction (Rendeiro, 2012). Thus, ensuring that good eating habits early in life with regular consumption of fruits and vegetables could result in better cognitive outcomes later in life. One particular class of nutrients that have been avidly investigated in recent years are the flavonoids, found in high concentrations in a number of fruits and vegetables. Diets rich in flavonoids have increasingly been shown to have positive effects on human health and therefore, may play a crucial role in maintaining long-term cognitive health.

Flavonoid-rich foods can improve memory and learning performance. Letenneur conducted a 10 year study in a sample of 1640 older adults aged 65 and over and found that participant's total flavonoid consumption was significantly correlated with their cognitive ability (Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-Gateau, 2007). Not only do, diets rich in flavonoids produce benefits to cognition, they have also shown positive effects on cardiovascular function in humans (Schroeter, 2006). Indeed, flavonoid-rich cocoa has been found to enhance cortical blood flow (Francis, 2006; Fisher, 2006). This result is crucial as the improvement of cerebrovascular performance, particularly in the hippocampus (a brain region critically involved with learning and memory) may result in adult neurogenesis (Gage, 2000). During new neuronal growth, neuronal spine density and morphology increased and these changes are crucial for learning and memory. Indeed, a study by Palmer showed that new hippocampal cells were found located close to blood vessels, proliferated in response to vascular growth factors, and could directly affect cognitive performance (Palmer, 2000). Thus, supplementing the normal diet with foods and beverages (derived from grape, tea, cocoa, and blueberry) that are rich in flavonoids may, therefore, impact on cognitive function. A number of studies in adults have shown benefits to cognitive performance and mood from diets rich in flavonoids, however much less evidence exists for school-aged children. The observed positive effects of flavonoids in adults on attention and memory processes, if replicated in children, would have significant implications for cognitive development and may prove particularly beneficial in an educational setting.

1.2 Basic concept of Flavonoids

1.2.1 Structure of flavonoids

Flavonoids are phytochemicals synthesized by plants and are part of a large family of polyphenolic compounds. The structure of Flavonoids are C_6-C_3-C_6, which are linked two benzene ring (ring A and B) linked by a heterocyclic pyrane ring (ring C) (see in Figure 1-1). A number of different sub-groups of flavonoids exist, determined by the degree of saturation of the C-ring and the hydroxylation of the ring structure. As shown in Figure 1.1, the six mjor sub-classes of flavonoids are: 1) flavonols; 2) flavonones; 3) isoflavonones; 4) flavanones; 5) flavanols (or flavan-3-ols or catechins); and 6) anthocyanidins.

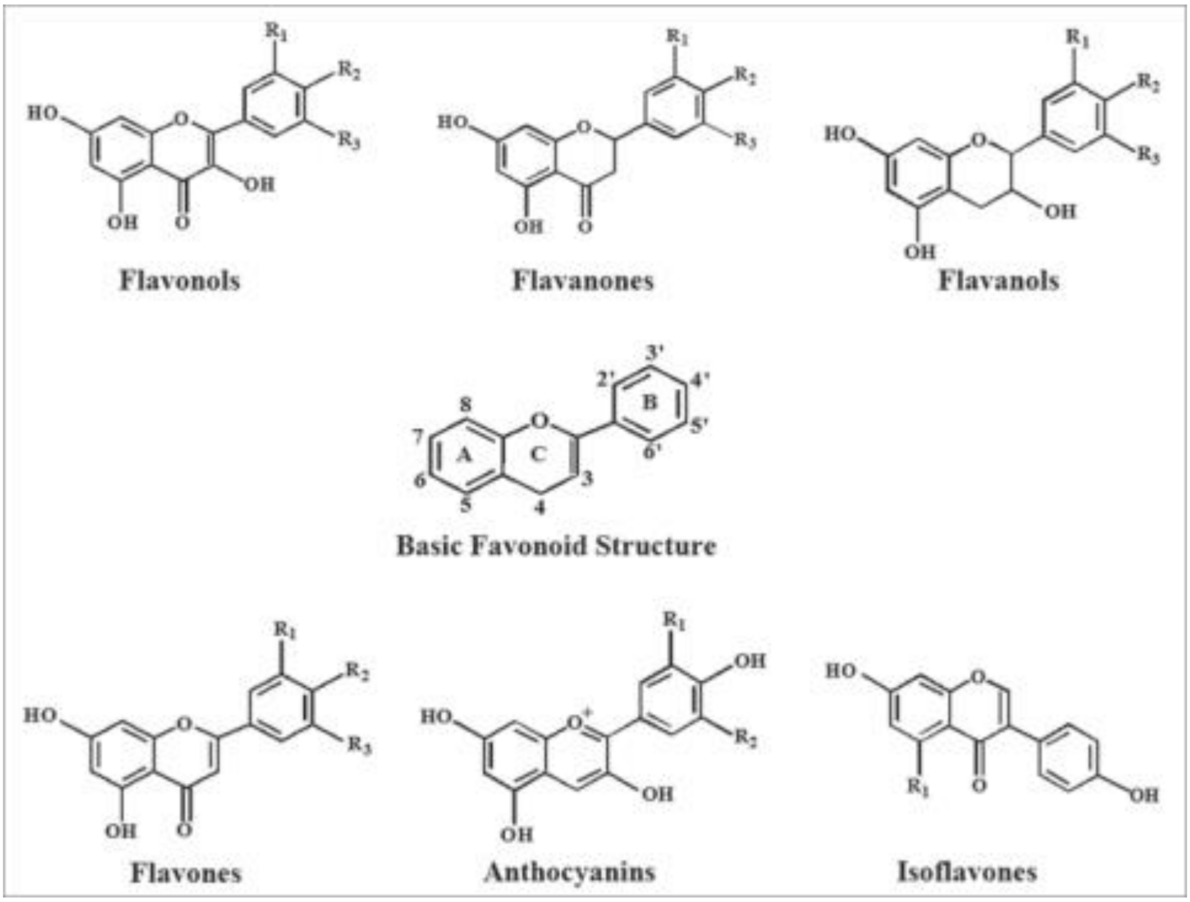

Figure 1-1 Structure of flavonoids, showing basic flavonoids structure (middle) and the other flavonoids subclass (adapted from Kanti Bhooshan Pandey (2009)).

1.2.2 Sources of flavonoids

Fruits, vegetables, tea, wine and fruit juices are the main dietary sources of flavonoids with particular high concentrations of sub-classes of flavonoid found in certain foods. Typically, flavan-3-ols are commonly present in fruits (except citrus), apple peels, berries, cherries, grapes, plums, apricots, red wine, chocolate and teas (particularly green, white, black and oolong). Flavonols can be found in vegetable such as onions, curly, kale, leeks, broccoli, celery, capers, fennel, buckwheat, and in fruits such as apricots, bilberries, elderberries, currents, cranberries, blueberries and also found in beverage such as tea, red wine and cocoa powder. Anthocyanins are naturally found in blue, purple or red colours of plant, which are most abundant in blackberries, black current, blueberries, black grapes, strawberries, cherries, as well as some vegetables such as cabbage, beans, onions, radishes and also in red wine. Isoflavones can be primarily found in leguminous plants and soy. Flavanones are commonly found in citrus fruits and juices such as oranges, grapefruits and lemons. The last subgroup and least common, flavones are mainly present in celery, parsley, thyme and hot peppers.

1.2.3 Metabolism

A number of papers, such as Thilakarathna and Rupasinghe (2013) and Rodriguez-Mateos et al. (2014), have fully described the process of flavonoid metabolism once ingested. Briefly, that flavonoids are initially modified by hydrolysis from saliva in the oral cavity. Ingested flavonoids then undergo further modification in the gastrointestinal tract as they transit through the stomach, small intestine and colon. In the small intestine, flavonoids, in the form of glycosides interact with lactase phloridzin hydrolase (LPH) to form aglycones. Aglycones are then absorbed and transported to the liver for further metabolism. Phase I and II metabolism of the aglycones occur in both small intestine and liver where they are conjugated with sulphates, glucuronic acid and methylate. Metabolites in the liver then enter the circulation and eventually undergo renal excretion. Some metabolites from the liver can be recycled back to the small intestine via enterohepatic recirculation in bile. Any unabsorbed compounds from the small intestine travel down to the colon where the resident microflora degrade flavonoids into low molecular weight catabolites (phenolic acids) that can be absorbed by the small intestine or the liver where further phase I and II metabolism can occur. A diagram showing flavonoid metabolism can be seen in Figure 1-2.

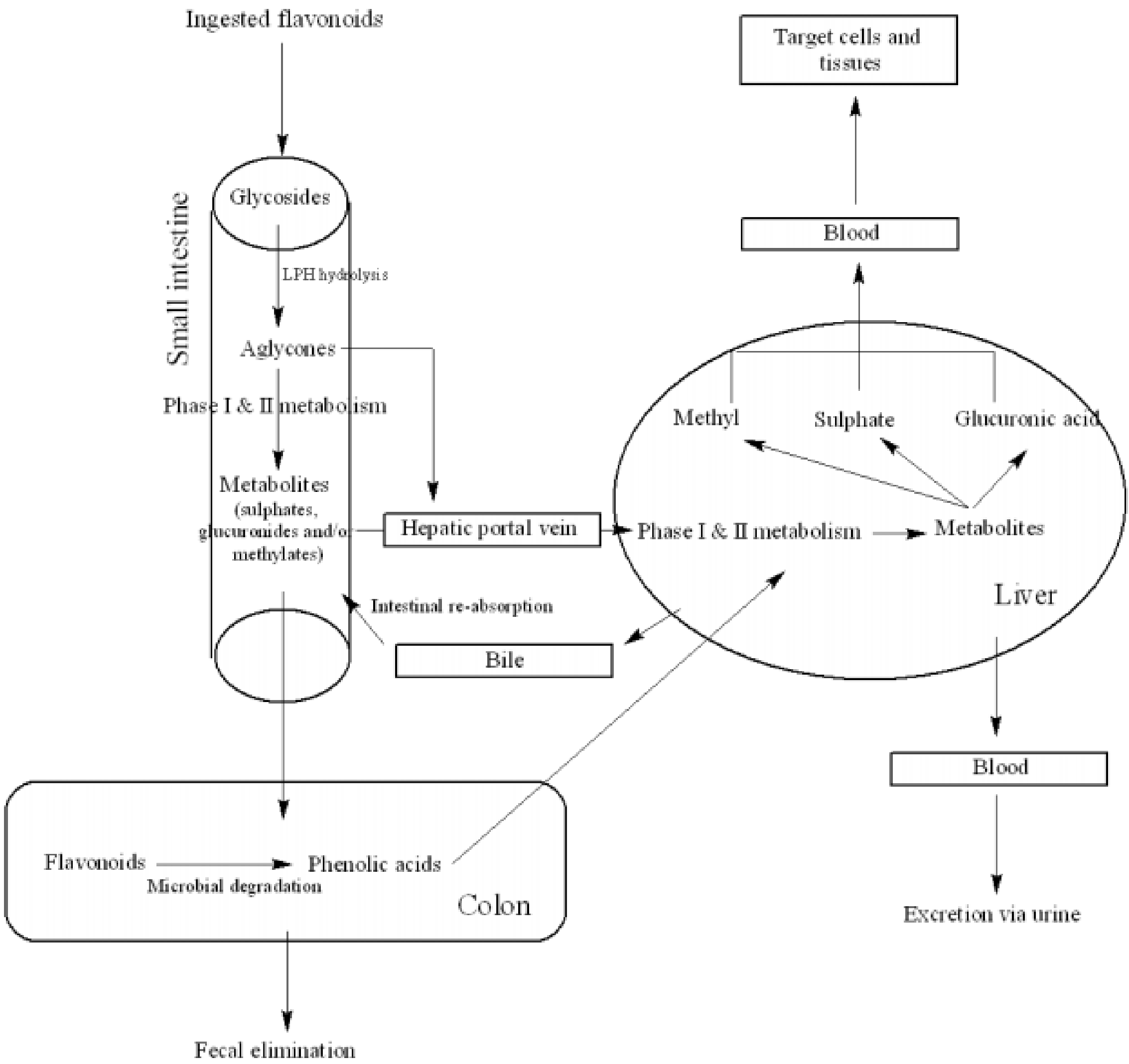

Figure 1-2: Flavonoids Metabolism in the body (adapted from Thilakarathna and Rupasinghe (2013)).

1.2.4 Mechanisms

The possible mechanisms by which flavonoids impact within the brain to enhance memory, learning and cognitive performance has recently been described. Spencer (2010) documented that flavonoids and flavonoid-rich fruits may influence innate cellular structures and in this way, affect cognitive processing via three major mechanisms. Firstly, by interacting with neuronal signalling and synaptic functions they can induce an improvement in neuronal communication and increase neuronal survival and differentiation. Secondly, by influencing blood flow, they can improve endothelial function and result in neurogenesis. Finally, by inhibiting neurodegeneration and neuroinflammation whereby flavonoids exert a neuroprotective effect by inhibiting cytokine release from activated glia, protect iNOS induction and nitric oxide production from glial activation, prevent the NADPH oxidase activation and ROS generation in activated glia, and reduce pro-inflammatory transcription factors activity (Spencer, Vafeiadou, Williams, and Vauzour (2012).

1.3 Flavonoids and Health

Recently, there has been increasing interest in the health benefits of polyphenols, particularly flavonoids. It can be seen from numerous epidemiological and clinical studies that long-term consumption of flavonoid shows an inverse association with the risk of chronic disease such as cancer, cardiovascular diseases and neurodegenerative diseases. In the section below, I will present a brief overview of the beneficial effects of flavonoids on human health.

1.3.1 Cardiovascular Disease (CVD)

Flavonoids may act as potent inhibitors of LDL oxidation, which is the major cause of atherosclerosis and cardiovascular diseases. In addition, their antioxidant, anti-platelet, anti-inflammation actions, as well as actions to enhance HDL and promote endothelial function may also prevent cardiovascular disease (Garcia-Lafuente, Guillamon, Villares, Rostagno, & Martinez, 2009).

Several studies have demonstrated the relationship between flavonoids consumption and the risk of coronary heart disease (CHD). One systematic study reviewed the six classes of flavonoids and showed that intake of flavonols, anthocyanidins, proanthocyanidins, flavones, flavanones and flavan-3-ols significantly reduced the risk of cardiovascular disease (CVD) (Wang, Ouyang, Liu, & Zhao, 2014). Furthermore, in men with an increased risk of CVD, those participants that consumed a diet rich in flavonoid-containing fruits and vegetable had increased endothelium-dependent microvascular reactivity and plasma NO and also reduced C-reactivity protein, E-selectin and vascular cell adhesion which are the protective against vascular disease (Macready et al., 2014). Indeed, a long-term prospective cohort study recently demonstrated that flavonol intake was significantly associated with lower risk of CVD incidence. Indeed, after adjustment for age, gender, body mass index, smoking and energy intake, there was a significant negative association of high flavonol intake with lower risk of CVD. This study interpreted that flavonol intake may be most strongly associated with CVD risk than any of the other subclass of flavonoids or other forms of diets. (Jacques, Cassidy, Rogers, Peterson, & Dwyer, 2015). A study of 38,180 men and 60,289 woman in the Cancer Prevention Study II Nutrition Cohort assessed mortality from CVD in men and woman over a seven year period. They found that men and women in the top quintile of flavonoids consumption had a lower fatal CVD risk. Analysis of the individual subclasses of flavonoids showed that only total flavonoids intake in men was associated with stroke mortality and ischemic heart disease, whilst flavone intake had strongest association with ischemic heart disease (IHD) mortality in woman. Furthermore, this study showed that even a low flavonoid intake may prevent CVD death rate (McCullough et al., 2012). Finally, a study of total dietary intake and chronic diseases found that mortality from ischemic heart disease was inversely associated with the consumption of quercetin, and a lower incidence of CVD was associated with higher consumption of kaempferol, naringenin, and heperetin (Knekt et al., 2002).

1.3.2 Cancer

Flavonoids have also been ascribed roles in combatting cancer, e.g. chemoprevention activity, prevention of oxidation, induction of detoxification of enzymes, anti-inflammatory and promotion of host immune system (Rodriguez-Mateos et al., 2014).

A number of epidemiological studies have demonstrated an inverse association between flavonoid intake (from fruit and vegetable) and the incidence of various cancers. A meta-analysis of six prospective cohort studies and six of case-control studies demonstrated that women with high intake of flavonols and flavones had a negative association with breast cancer. No association was observed for intake of flavan-3-ols, flavanones, anthocyanins or total flavonoids with breast cancer risk (Hui et al., 2013). The positive effect of flavonoid consumption and cancer have also been shown in a study of flavonoid intake and chronic diseases in Finland where men who higher consumption of quercetin had a lower incidence of lung cancer, and those who had a higher consumption of myricetin had lower prostate cancer risk (Knekt et al., 2002). Similarly, one further study of flavonoids intake and cancer risk, this time in postmenopausal women from Iowa showed that recent or past smokers with high intake of flavanones and proanthocyanidins had a negative association with lung cancer incidence rate (Cutler et al., 2008). Whilst, a prospective study of Nurses health showed that those with the highest quintiles of flavonol and flavanone consumption had a moderately lower risk of ovarian cancer comparison with the lowest quintiles, this just failed to reach significance (Cassidy, Huang, Rice, Rimm, & Tworoger, 2014).

However, not all data shows a relationship between high flavonoid intake and lower cancer risk. Two large prospective studies from the 'Health Professionals Follow-Up Study' and from the 'Nurses' Health Study' have found no statistically significant relationship between intake of any flavonoids subclasses and the risk of colorectal cancer (Nimptsch et al., 2016). The inconsistency of results in this field may, however, be attributable to differences in sociodemographic, lifestyle and agricultural factors of the population and also the dose and timing of flavonoid consumption may affect the anticancer actions from flavonoids rich diet (Romagnolo & Selmin, 2012).

1.3.3 Neurodegenerative Disease

Neuroinflammatory processes may cause a number of neurodegenerative diseases such as Parkinson's (PD), Alzheimer's (AD and Huntington's diseases, and amyotrophic lateral sclerosis (ALS)(Mandel, Weinreb, Tamar Amit, & Youdim, 2004). The anti-inflammatory, antioxidant and metal-chelating properties of flavonoid-rich diets may therefore, counteract the neuroinflammatory process. A systematic review of nine cohort studies assessing fruit and vegetable intake and prevention of cognitive decline or dementia, showed 6 studies reported participants with a high consumption of vegetables (but not fruit) and lower risk of dementia, whilst the remaining 3 studies showed that intake of both fruits and vegetables suppressed dementia risk (Loef & Walach, 2012). In the Scarmeas study of the Mediterranean diet (a diet with high intake of fruits, vegetable, legumes, cereals, uncaptured fatty acids (olive oil) but with a low intake of saturated fatty acids) was associated with a lower risk of Alzheimer disease (Scarmeas, Stern, Tang, Mayeux, & Luchsinger, 2006). Similarly, a large cohort study assessing flavonoid intake and dementia risk showed that, during a 5 year follow-up, elderly subjects with highest intake of flavonoids had the lowest risk of dementia compared to those participants with lowest flavonoid intakes (Commenges et al., 2000).

1.4 Flavonoids in Thai Fruits

1.4.1 Fruit and Vegetable consumption in Thailand

Over the last two decades, Thailand has experienced significant economic change. The main economic structure has changed from traditional agriculture-base to industrially- and commercially-based activities. At the same time, social structure and lifestyles also have shifted from rural to more urban societies. These changes in lifestyle have impacted significantly upon the typical Thai diet, with diet patterns changing from a traditional carbohydrate base which contained plenty of rice, fruit and vegetable but minimal meat intakes, to a modern diet higher in fats and sugars. However, intakes of fruit and vegetables are still significantly lower than recommendations from the World Health Organisation (WHO).

To address these issues, Thailand has relatively recently initiated a dietary campaign, called the Thailand Nutrition Flag, aimed at promoting consumption of healthy foods containing adequate amounts of fruits and vegetables. This campaign recommends 4-6 servings of vegetables and 4 servings of fruit per day, amongst a number of other dietary recommendations (see Figure 1.3). On the basis of these recommendations, many studies have shown that individuals, across the age range, consumed inadequate daily fruit and vegetable intakes (Working group on food-based dietary guidelines for Thai people: quantitative process. Institute of Nutrition, 2001).

Figure 1-3: The Thailand Nutrition Flag provided dietary guidelines to encourage individuals to eat healthily.

Indeed, the 4th National Nutrition Survey in 1995, which used 24-hour dietary recall to collect intake data of more than 106 kinds of various vegetables and more than 9 kinds of various fruits, reported that Thai people consumed relatively low amounts of fruit and vegetable (Ministry of Public Health (MOPH), 1995). Here, Thai people ate an average of 113.2 grams of vegetable per person per day equating to only 2 servings, and 73.6 grams of fruit per person per day equating to only 1-2 servings (total fruit and vegetable intake was 186.8 grams per day per person). This is significantly below the WHO recommendations.

Similarly, the study of the Physical Activity Division, Ministry of Public Health (MOPH, 2000) evaluated health behaviours and physical activity practices among MOPH employees. The result found that almost 50% of their sample, particularly employees with higher education degrees and socio-economic status represented by civil servants, consumed only 2 servings of fruit and vegetable per person per day (equivalent to 135 grams). This was in contrast to research by Satheannoppakao, Aekplakorn, and Pradipasen (2009) who examined the consumption of fruit and vegetable in Thai people using a short semi-qualitative FFQ administered to both community-dwelling men and women aged ≥ 15 years. Here they report that average daily intake of fruit and vegetable was 3.24 serving per person per day.

In the Thailand National Health Examination Survey III, conducted in 2003-2005, they reported that Thais aged 15 years and over consumed vegetable and fruits below the daily recommended requirement for health promotion and disease prevention. Here, men ate 268 g/day and women ate 283 g/day. Importantly, levels of fruit and vegetable consumption were found to be significantly decreased as Thai people got older with, for example, the 80 year olds and over having the lowest intake at about 200 g/day per person (Ministry of Public Health, 2006). Sociodemographic characteristics also affect fruit and vegetable consumption amongst the Thai population with higher intake of fruit was associated with being female, a secondary and vocational school level of education and a household income of 50,000 baht or higher per month. Higher vegetable consumption was associated with a household income of 50,000 baht or higher per month, and being a Bangkok resident.

Another study conducted by Peltzer and Pengpid (2012) investigated consumption of fruits and vegetables in school-aged adolescents in five Southeast Asian countries. The results found that Thai adolescents consumed the highest proportion of fruit and vegetable (3.7 servings per day) in comparison with adolescents from India, Indonesia, Myanmar and Sri Lanka adolescents who consumed on average of 3.0, 3.2, 2.9 and 3.1servings per day respectively.

To summarise Thai people consumed inadequate daily fruit and vegetable with average 1-3.7 serving per day, which is lower than WHO recommendations for 5 serving per day. The factors associated with consumption were education level, household income and residential areas.

1.4.2 Utilization of fruit and vegetable

Fruits and vegetables are consumed in many different ways in Thailand. Uncooked vegetables are commonly used as a side dish, whilst the cooked forms of vegetables are normally consumed as a side dish or mixed with meat, fish and other foods to form the main meal. The Thai meal commonly consists of rice, cooked vegetable and a curry dish. Vegetables are either consumed raw or cooked with water, oil, coconut milk then served with traditional chili sauces. A common meal among low-income families consists of plain or glutinous rice, traditional chili sauces and dry fish or roast chicken, all consumed with vegetables.

Thailand has a tropical climate, and it produces a large number of fruits and vegetables. The types of vegetable that Thais typically consume are leafy vegetables, such as amaranth, spinach, piper, sweet basil, carrot, awl tree, torvum, eggplant, and acacia shoot. When considering those vegetables rich in flavonoids, onion, Thai copper pod, tummy wood, penny-wort, coral tree, lead tree, balsam pear, and banana (flower) are commonly consumed (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). Types of vegetable that are high in flavonoids and regularly consumed in Thailand is shown in Table 1.1 below.

Table 1-1 Some Vegetables from Thailand that are rich in flavonoids

Common Name	Total flavonoids (mg RE/g db plant)
Onion	20.20
Thai copper pod (Flower)	24.80
Tummy wood (Young Leaves and Leaves)	20.50
Penny-wort (Young Leaves and Leaves)	25.50
Coral tree (Leaves)	20.00
Lead tree (Young Leaves and Leaves)	22.30
Balsam pear (Bud and Leaves)	21.60
Banana (Flower)	20.30

Thailand also produces a wide variety of fruit, not only for domestic consumption, but also for worldwide export. Typical fruits in the Thai diet include mango, papaya, mangosteen, banana, durian, guava, pomelo and pineapple. There is much less data available about flavonoid content of typical fruits from Thailand, however a study by Kongkachuichai, Charoensiri, and Sungpuag (2010) studied the carotenoid and flavonoid profiles, as well as the dietary fibre content of some commonly consumed Thai fruit. The results show that durian, pomelo, guava and ripe banana were good sources of flavonoids, with the Thong dee and Tuptimsayam variety of pomelo demonstrating the highest total flavonoid content (13,994.21 and 15,094.99 µg per 100 g fruit portion). Other kind of fruit that presented high flavonoid contents are guava (Pan see thong variety; 8,926.69 µg per 100 g fruit portion), ripe durian (Mhon thong variety; 4,485.18 µg per 100 g fruit portion) and pineapple (Phuket variety; 4,268.64 µg per 100 g fruit portion). Flavonoid content of commonly consumed fruits in Thailand is shown in Table 1.2 below.

Table 1-2 Flavonoids contents of fruits commonly consumed in Thai

Common Name	Total flavonoids (µg /100 g edible portion)
Ripe banana	
Khai	3,560.53
Nam-wa	3,376.52
Leb mu nang	3,057.73
Hom	4,063.63

Common Name	Total flavonoids (µg /100 g edible portion)
Ripe durian	
Kan yao	3,508.47
Kradum	4,480.74
Chanee	1,904.58
Puang manee	3,233.95
Mho thong	4,485.18
Guava	
Kim chu	3,168.04
Pan see thong	8,926.69
Ripe papaya	
Holland	916.00
Khak dahm	1,509.69
Mangosteen	1,515.18

Common Name	Total flavonoids (µg /100 g edible portion)
Pomelo	
Khao tang kwa	7,368.47
Khao num phung	8,939.70
Thong dee	13,994.21
Tuptipsayam	15,094.99
Pineapple	
Phuket	4,268.64
Sri racha	4,187.96

1.5 Summary of epidemiology, acute and chronic studies in flavonoids and cognition

Over the last few years, a growing number of studies have shown positive associations between consumption of whole fruits and better cognitive performance. Epidemiological studies have demonstrated that fruit consumption leads to better cognitive evolution (Butchart et al., 2011; Devore, Kang, Breteler, & Grodstein, 2012; Letenneur et al., 2007; Nooyens et al., 2011; Nurk et al., 2010; Polidori et al., 2009; Ye, Bhupathiraju, & Tucker, 2013), whilst further evidence from both acute and chronic intervention studies using fruit also show boosts in cognitive performance (Alharbi et al., 2015; Bondonno et al., 2014; Kean et al., 2015; D.J. Lamport et al., 2015; Mastroiacovo et al., 2015; A. W. Watson et al., 2015; Whyte, Schafer, & Williams, 2015; Whyte & Williams, 2015). In the following section, I will review the evidence surrounding impact of fruit on cognition.

1.5.1 Evidence from animal studies

As my studies will focus on supplementation to humans, I will only briefly review the evidence from the animal literature showing positive effect of flavonoid supplementation on cognitive performance. One of the seminal studies in this field was the study by Joseph and colleagues (Joseph et al, 1999) who demonstrated a reversal of age-related deficits in a number of motor and cognitive tasks following 8 weeks supplementation of strawberry, spinach, or blueberry extract in 19 month old Fischer rats. This work was followed by a sequence of studies from the Williams lab showing upregulation of critical signaling proteins involved in hippocampal neurogenesis following flavonoid-rich blueberry treatment (Rendeiro et al., 2014; Rendeiro et al., 2013; Williams et al., 2008). Williams et al. (2008) supplemented 2% blueberry to the diet of 18 month old Lister-hooded rats for 12 weeks and found significant improvements in spatial working memory. Here, in comparison to an age-matched group fed a standard diet, the rats supplemented with blueberry (267.2 μg/g anthocyanins and 153.9 μg/g flavanols) showed better performance in the number of correct choices and time required to make a choice after only 3 weeks of supplementation. The improvement in rats with blueberry supplementation were sustained for the remaining 9 weeks of the testing period, and were comparable to the performance seen with 6 month old young rats fed a control diet. Following on from this work, Rendeiro et al. (2013) supplemented groups of 18 month old rats for 6 weeks with one of four diets: a control diet, 2 % blueberry diet, an anthocyanin-rich diet (equivalent to the amount of anthocyanin found in the 2% blueberry diet) and a flavonol-rich diet (again equivalent to the amount seen in the blueberry group). A cross maze task was again used to measure spatial working memory. Better performance was seen for all groups supplemented with a flavonoid-rich diet. Finally, Rendeiro et al. (2014) also supplemented young healthy rats with 3 weeks supplementation of 8.7 mg per day and 17.4 mg per day of blueberry powder from Vaccinium Corymbosum high-bush blueberries. Here, both groups showed an improvement in spatial learning and memory. Importantly, both of these studies (Rendeiro et al, 2013; Rendeiro et al, 2014) demonstrated that the improvement of memory was associated with increased cAMP-response element-binding protein (CREB) activation in the hippocampus alongside, increased extracellular signal-related kinase (ERK 1/2) and pro-and mature level of brain-derived neurotropic factor (BDNF) activity.

Further evidence showing benefits of flavonoid supplementation to laboratory animals have been reported by numerous laboratories who have shown improvement of learning and memory following berry fruit supplementation (Willis, Shukitt-Hale, & Joseph, 2009 supplementation with blueberry or strawberry extracts (Shukitt-Hale, Carey, Jenkins, Rabin, & Joseph, 2007), polyphenol-rich oriental plum (K. Lee et al., 2013), concord grape juice (Shukitt-Hale, Carey, Simon, Mark, & Joseph, 2006), blackberry (Gomar, Hosseini, & Mirazi, 2014), and cherry (Thangthaeng et al., 2016).

The evidence from these animal studies all strongly support the notion that flavonoids are associated with improvements in learning and memory, potentially due to their neuroprotective actions on cell signaling molecules.

1.5.2 Evidence from epidemiology studies

Various epidemiological studies support the potential health benefits of a flavonoid-rich diet in protecting against neurogenerative disease and decelerating the rate of cognitive decline. Letenneur et al. (2007) conducted a prospective epidemiologic study to examine the effect of flavonoids intake on cognitive ability over a 10-year period. The participants were 1,640 older adults aged 65 years or older free from dementia at the beginning of the study. The Mini-Mental State Examination (MMSE), Benton's Visual Test and the "Isaacs" Set test were employed to assess cognitive ability at four individual time points during the 10 years of the study. Flavonoid intake at baseline, after adjustment for age, gender, education level, body mass index, smoking status and fruit and vegetable intake, was positively associated with better cognitive scores, as well as better cognitive evolution over time. Furthermore, after 10-years follow-up, participants with the lowest quartile of flavonoid intake had shown a decrease MMSE score of 2.1 points compared to a decrease of only 1.2 points for those participants in the upper quartile of flavonoid intake. The result of this study provides important evidence that flavonoids, particularly from fruits and vegetables, show positive neuroprotective effects, promote a better general cognitive performance and also protect against cognitive decline in the elderly.

Another study supporting the effect of flavonoids to alleviate cognitive decline was reported by Nurk et al. (2010) who examined the association between consumption of plant-based foods and cognitive performance of elderly participants aged 70-74 years in the Hordaland Health Study, Norway. The Kendrick Object Learning Test, Trail Making Test-part A, modified Digit Symbol Test, Block Design, Mini-Mental State Examination and Controlled Oral Word Association Test were used to measure cognitive performance. The participants also completed a self-report FFQ to demonstrate habitual food consumption. The participants with higher intake of fruits, vegetables, grain products and mushrooms performed better on the cognitive tasks compared to those with low or no intake of those foods. Fruits and vegetable combined were most strongly associated with cognitive performance particularly for those individuals with intake of 500g or more per day. For individual foods, there was a positive association between cognition and intake of carrots, cruciferous vegetables, citrus fruits and high-fibre bread. The only negative cognitive association was found for high consumption of white bread. Nurk et al. (2009) also conducted a cross-sectional study to examine the association between cognition and three commonly consumed high-flavonoid foods. Using the same cognitive test battery as described in the previous study (Nurk et al., 2010), they found that intake of chocolate, wine and tea positively influenced cognitive performance, with those individuals having high intake having significantly higher mean test scores and lower rates of poor cognitive ability relative to those who consumed less of these foods. The effect was most strongly associated with intake of 75-100 ml per day for wine intake and moderately with 10 g per day of chocolate intake. Both these studies (Nurk et al., 2009, 2010) strongly support the evidence that high consumption of rich flavonoids foods is related with higher cognitive ability.

The association between fruits and vegetable consumption and cognitive decline was also repeated by Polidori et al. (2009) who used 193 participants to examine whether high intakes of fruit and vegetable had positive effects on cognitive function. Mini-Mental State Examination (MMSE), Clock Drawing Test, and the DemTect Scale were used to measure cognitive function while FFQ was used to estimated daily fruit and vegetable consumption. The participants with the highest fruit and vegetable intake performed better on the cognitive tasks than those participants with lower intakes.

However, a number of studies that analysed intake of fruit, vegetable and fruit and vegetable combined have shown some inconsistent results. Morris, Evans, Tangney, Bienias, and Wilson (2006) conducted a prospective cohort study to evaluate the association between age-related cognitive decline and fruit and vegetable intake. FFQ was used to assess fruit and vegetable intake, and East Boston Tests of immediate memory and delayed recall, Mini-Mental State Examination, and Symbol Digit Modalities Test were used to measure cognitive change. Here, those individuals with the highest quintile of vegetable intake, relative to those with lowest quintile after adjustment of confounding factors, showed the slowest rate of cognitive decline. No significant association with fruit intake was shown. The effect of vegetable, but not fruit, to reduce rates of cognitive decline has also been shown by Kang, Ascherio, and Grodstein (2005) and Pastor-Valero et al. (2014).

Nooyens et al. (2011) examined the cognitive decline and dementia in 2613 elderly participants in the Netherlands. The cognitive tests consisted of global cognitive function and then specific assessment of the domains of memory, information processing speed and cognitive flexibility. Cognitive performance was assessed at baseline and then twice every 5 years. Habitual fruit and vegetable consumption was measured by semi-quantitative food frequency questionnaire. At baseline, participants with higher vegetable intake showed a significant association with lower information processing speed and worse cognitive flexibility, however at the end of the study, these participants showed a smaller decrease in processing speed and global cognitive than other participants. In term of total fruit intake, legumes and juices showed no association with any cognitive function at both baseline and follow-up. Interestingly, higher intake in some specific fruit and vegetable, particularly, nuts, cabbage and root vegetable showed better cognitive ability at baseline and smaller reduction at follow-up.

Using a prospective study, Peneau et al. (2011) measured the effect of fruit and vegetable intake on cognitive ability as part of the Supplementation with Antioxidant Vitamins and Minerals 2 (SU.VI.MAX 2) study. Fruit and vegetable consumption were assessed using 24-h dietary records, whilst cognitive function was measured at baseline and after 13 years using composite verbal memory (from RI-48 cued recall, semantic, and phonemic fluency tests) and composite executive function score (from trail-making and forward and backward digit span tests). In term of composite memory score, fruit and vegetable combined and fruit intake alone were shown to have a positive association with better cognitive function, whereas, they found an unexpected negative association between fruit and vegetable combined and vegetable intake alone on composite executive function score.

However, a number of studies also show no association between fruit and vegetable intake and cognition. Ye et al. (2013) reported that it is not the total amount of fruit and vegetable consumed but rather the variety of fruit and vegetables that had a positive association with better MMSE score, executive function, memory and attention. This cross-sectional study included 1412 middle-aged and older Puerto Rican adults assessing fruit and vegetable consumption using the FFQ, measuring cognition using 7 different cognitive tasks and MMSE to measure global cognitive score. The authors suggest that the wider varieties of fruit and vegetable may result in intake of more varied bioactive substances and the combination of these may have synergistic actions on the pathophysiological process of cognitive decline.

Further evidence by Butchart et al. (2011) have shown inconsistent results or no effect of fruit and vegetable intake and cognitive function. This cross-sectional study included 1091 elderly British aged 70 years old. Childhood IQ was measured at the aged of 11 (taken from the Lothian Birth Cohort 1936 study) and then various neuropsychological tests and FFQ were investigated at the aged of 70 years. Here, there was an association between total fruit, citrus fruits, and apple and tea consumption with better cognitive test scores, however this association disappeared when childhood IQ score was controlled for. Interestingly, flavanone showed a positive association with better verbal fluency scores, which again was lost after controlling for childhood IQ. Similarly, a study by Yuan et al. (2014) using 504 community dwelling elderly aged between 55 to 75 specifically investigated dietary flavonoids intake and cognitive function. Food frequency questionnaire was employed to assess habitual food intake and the Montreal Cognitive Assessment was employed to investigate cognitive function. Here, more frequent fruit and vegetable intake produced significant effects on levels of antioxidant biomarkers, however this did not boost cognitive ability.

Finally, Devore et al. (2012) studied the relationship between long-term berry intake (anthocyanidins) and rates of cognitive decline in 16,010 nurses. A semi-quantitative food frequency questionnaire was assessed at baseline and every 4 years while cognitive function was measured at baseline and then every 2 years. Z score of all cognitive test including telephone Interview of Cognitive Status, a telephone adaptation of the Mini-Mental State Examination; East Boston Memory Test – immediate and delayed recalls; category fluency; delayed recall of the Telephone Interview of Cognitive Status 10-word list; and digit span backward were calculated as composite global score, while the immediate and delayed recalls of both the East Boston Memory Test and Telephone Interview of Cognitive Status 10-word list were calculated as composite verbal score. After controlling the confounds of age and education status, the participants with the highest intake of blueberries and strawberries had better global cognitive score, in comparison to participants with lower intakes of these berries. These results again support the concept that high consumption of flavonoid can protect against cognitive decline.

In summary, the evidence from epidemiology studies demonstrate some consistency showing that high fruit and vegetable consumption produces a significant impact on better cognitive performance, especially in elderly participants. However, due to the complexity and cost of performing such studies, there are no epidemiological studies focusing on younger age groups or children and the impact of their diet to protect against neurogenerative diseases in the future.

1.5.3 Evidence from chronic supplementation study in adults

A large number of studies have assessed the effects of cocoa interventions on cognitive performance. Camfield et al. (2012) conducted a randomized, double-blind placebo-controlled trial in 63 participants aged between 40 to 65 year-old consuming either a high- (500 mg), low-flavanol (250 mg) or placebo cocoa drink 5 for 30 days. Steady state visually evoked potential (SSVEP) topography was used to image neurocognitive change concurrently with performing a spatial working memory task. Interestingly, there were no significant effects on the participant's accuracy and reaction time in the task itself however, during memory encoding, there were significant differences in average SSVEP amplitude and phase differences at a number of posterior parietal and centro-frontal sites between the two conditions. Specifically, the placebo group showed an increase in SSVEP amplitude in posterior-parietal regions during encoding but SSVEP amplitude increased in frontal and posterior-parietal regions during the hold and retrieval phases. In comparison, SSVEP amplitude in the consumers of the low flavanol group was lower in posterior-parietal regions during the hold and encoding intervals with a slight increase during the retrieval interval. Finally, for the high flavanol group, SSVEP amplitude was slightly decreased in posterior-parietal regions during all phases. As no behavioural differences were seen, interpretation of these data is somewhat limited however due to the difficulty of the SWM task in this study was moderate, it is possible that chronic cocoa flavonol consumption may improve neural activity in spatial working memory function if the task was made more difficult to detect performance.

Utilising a longer duration of study, Crews Jr, Harrison, and Wright (2008a) conducted a 6-week intervention using 101 participants aged sixty years old or more. Participants were administered either a dark chocolate bar and an artificially sweetened cocoa beverage (containing 397.30 mg proanthocyanidins from the chocolate bar and 357.41 mg from cocoa drink) or similar placebo products (containing 0.20 mg proanthocyanidins from the chocolate bar and 40.87 mg from cocoa drink) each day for 6 weeks. There were no significant differences in performance between the two groups on the cognitive tasks (including selective reminding test, Wechsler Adult Intelligence Scale-III, Wechsler Memory Scale-III, TMT and Stroop Colour-Word Test.). However, consumption of dark chocolate and cocoa was associated with significantly higher pulse rates at 3- and 6-wk treatment assessments.

Mastroiacovo et al. (2015) conducted an experiment to evaluate the effect of 8-weeks flavonoid supplementation on cognitive performance in older adult aged 90 with cognitive dysfunction. Using a double-blind design, participants were allocated into one of three groups allocated to consume 993 mg (high flavonoid; HF), 520 mg (intermediate flavonoid; IF) and 48 mg (low flavonoids; LF) cocoa drink for 8 weeks. Mini-Mental State Examination (MMSE), the Trial Marking Test (TMT) A and B, and the Verbal Fluency Test (VFT) were employed to measure cognitive performance at baseline and the end of the 8 week intervention period. Although there was no statistically significant difference in MMSE scores, the individual cognitive tasks did show some statistical difference between treatments. Both HF and IF groups were significantly quicker to complete the TMT-A and TMT-B, in comparison to the LF group. Whilst for VFT, the HF group performed better than either the IF or LF group. However, no placebo treatment was included in this experiment, and so results should be treated with caution.

Dietary flavonoids have also shown effects to enhance dentate gyrus function, an area of hippocampal circuitry associated with significant age-related change. Brickman et al. (2014) investigated the effects of 3 months supplementation with high- (900 mg of cocoa flavanols and 138 mg of (-)-epicatechin daily) or low-flavanol (10 mg of cocoa flavanols and < 2 mg of (-)-epicatechin daily) cocoa in 37 healthy 50-69 year-old participants. The participants were randomly allocated to one of four intervention groups, which included two high flavanol groups with or without a regimen of aerobic exercise, and two low flavanol groups who consumed with or without a regimen of aerobic exercise. The participants were imaged with cerebral blood volume (CBV)-fMRI and tested on aModBent task to measure the cognitive performance at baseline and the end of intervention. The secondary outcome was aerobic capacity (VO_2 max) that determined the effects of the aerobic fitness regimen. In addition, all participants completed a delayed retention task. The result revealed that the high flavanol condition, independent of exercise, showed a significant better performance on the ModBent task but had no effect on performance of the delayed retention task. Overall, participants in the high flavanol condition showed greater improvement in cognitive performance in comparison to those participants in the low flavanol condition. There were no effects of exercise on the ModBent task or on delayed retention. Finally, a significant improved of CBV in high flavanol participant was observed, which correlated with performance change on the ModBent task.

It is not only cocoa flavanols that have shown significant boosts in cognitive performance following chronic dosing. Kean et al. (2015) conducted an 8 weeks crossover, double-blind, fully randomized trial with 4 weeks washout between two flavanone conditions. The participants were 37 healthy older adults (average age 67) supplemented with either a high flavanone 100 % orange juice containing 305 mg flavanone (HF) or a low flavanone orange-flavored cordial containing 37 mg flavanone (LF). Cognitive performance was measured using the Go-NoGo, DSST and the letter memory to evaluate executive function, and the CERAD to evaluate episodic memory. Mood and blood pressure were also measured. The results demonstrated that participants in the high flavanone condition performed better on measures of global cognitive function (mean of all test combined) compared to those in the LF conditions, whereas no significant effect in individual score of executive function and episodic memory were found. However, no significant differences in mood and blood pressure were also observed.

More recently, a study by Kent et al. (2015) using 49 participants age over 70 year old with mild to moderate dementia has shown beneficial effects of long-term flavonoid treatment. Here, during a 12 weeks randomized controlled trial, participants were supplemented with either a 200 cc per day cherry juice (containing 138 mg of anthocyanin per day) or control drink (commercial apple juice containing 0.04 mg of anthocyanin per day). Participants were tested at baseline, 6 weeks and 12 weeks using the letter verbal fluency, category verbal fluency, RAVLT, trail making task, self-order pointing task, digit span backwards task, Boston naming task, and mood (Geriatric Depression Scale). Following supplementation with the anthocyanin-rich cherry drink, participants showed improvement in category verbal fluency (executive function), RAVLT total, RAVLT delayed recall and RVLT 20-min delayed recall compared to participants supplemented with the control drink.

To summarie, long-term supplementation with flavonoids show a consistent and positive effect on memory (Brickman et al., 2014; Kent et al., 2015; Pipingas et al., 2008), potentially due to their ability to increase neuronal activation in the posterior-parietal region in the brain (Camfield et al., 2012) and improve in cerebral blood flow (Brickman et al., 2014).It is not just the domain of memory that is boosted, flavonoid supplementation also enhances executive function (Kent et al., 2015; Mastroiacovo et al., 2015) and global cognitive score (Kean et al., 2015). However, a number of issues with the literature do exist as there is some inconsistencies in findings, and issues with replication of findings. It is often difficult to draw a comparison between different studies because of the differences in doses and treatments used. Furthermore, the length of supplementation in studies reviewed here is also variable with supplementation ranging from 4 weeks (Camfield et al., 2012) to 12 weeks (Kent et al., 2015), those studies with a shorter duration often have failed to show a significant behavioural effect (Camfield et al., 2012; Crews Jr, Harrison, & Wright, 2008b) but this is less apparent in those studies with a longer duration(Brickman et al., 2014; Kean et al., 2015; Kent et al., 2015; Mastroiacovo et al., 2015; Pipingas et al., 2008). The inconsistent cognitive effects of long-term flavonoid supplementation studies reviewed so far suggests that it is critical that future studies are well designed, have adequate sample sizes, proper dose and duration of supplementation in order to build a strong evidence to support the benefit of flavonoids on cognition.

1.5.4 Evidence from acute supplementation studies in adults

A number of acute flavonoids supplementation studies have also shown beneficial effects on cognitive performance. Alharbi et al. (2015) conducted an experiment using acute supplementation with a flavonoid-rich orange juice in males aged 30-65. Participants were randomly assigned to either an experimental group that consumed 240 ml orange juice (containing 272 mg of flavonoids), or a control group who consumed a calorie–matched placebo drink (containing no flavonoids). There was a 2 weeks washout between each drink. Eight individual cognitive tasks and a measure of mood were employed at the baseline, 2 hours and 6 hours post consumption. The result revealed that comparison to control drink the high flavonoid drink produced better performance on the individual cognitive tasks including simple finger tapping and Continuous Performance Task, however no significant differences between the two groups were seen for composite cognitive score and for mood.

Further evidence of cognitive improvement following acute citrus fruit supplementation was shown by D.J. Lamport et al. (2015) who conducted a crossover intervention study in twenty four young adults consuming a flavanone-rich orange juice (70.5 mg total flavonoids) or energy matched control drink. A vision task and cognitive tasks, including episodic memory, processing speed, working memory, and other executive functions, were utilised at baseline, 2 and 5 hours post consumption. Additionally, in a separate group of participants, fMRI was used to image cerebral blood flow (CBF) during the initial 2-hour post consumption phase. In comparison to the control group, the intervention group produced a significant improvement in performance on the digit symbol substitution task (indicative of quicker processing speed), however no other tasks reached significance. The result from fMRI showed that cerebral blood flow increased at 2 hr, but not at 5 hrs, following consumption of the high-flavanone drink. However, as the fMRI and cognitive test were not performed concurrently, it is not possible to confirm the association between improvement of processing speed and CBF change. In addition, it should be noted that the flavonoid content used in this study was low when compared to other studies, meaning that a higher dose may have elicited even greater boosts on cognitive performance.

A. W. Watson et al. (2015) conducted a double-blind, placebo-controlled crossover using blackcurrants, rich in anthocyanins, in young participants aged 18-35. The participants were provided with matched total polyphenol products (525 ±5 mg of polyphenols per 60 kg bodyweight) but different anthocyanin content from either a blackcurrant extract (483 mg of anthocyanin / 60 kg bodyweight), blackcurrant juice (467 mg of anthocyanin / 60 kg bodyweight), or 0 mg of polyphenol-matched control drink with at least 1-week washout between drink. Participants completed the digit vigilance, Stroop, rapid visual information processing (RVIP) and logical reasoning tasks, plus two mood scales at the baseline and 70 minutes after consumption. RVIP accuracy were improved after blackcurrant extract intake and increased reaction time of digit vigilance after blackcurrant juice intake. No significant difference in Stroop, logical reasoning, Bond-Lader visual analogue scales and Visual analogue scale were observed.

The beneficial cognitive effect of flavonoids can also be found from supplementation with other fruits. For example, Bondonno et al. (2014) examined the effects of acute supplementation with 4 conditions including 1) apple active (whole apple + skin containing 184 mg of total quercetin glycosides and 180 mg of (-)-epicatechin); 2) spinach active (containing 182 mg of nitrate); 3) apple and spinach combined; and 4) control rice milk (containing less than 5 mg of nitrate). This study hypothesised that the nitric oxide (NO) generated from supplementation with spinach and apple would increase blood flow and consequently improve cognitive performance. The Cognitive Drug Research (CDR) assessment battery and Bond-Lader mood scales were used to measure cognitive performance and mood at 2.5 hrs following supplementation. Participants treated with apple, spinach and the combination showed significant increases in plasma NO compared to control group but this did not result in increased performance on the cognitive tasks or boost mood. Even though the increase in NO from acute apple and spinach supplementation had no significant effect on cognition in this study, the possibility of cognitive improvement with long-term consumption cannot be ruled out.

Non-fruit based flavonoids, primarily cocoa flavanols, have also been widely used in several intervention studies. The flavanol, epicatechin, is the main flavonoid found in cocoa. Scholey et al. (2010) have demonstrated the beneficial of cocoa flavanol (CF) on cognition in thirty healthy adults, and this was the first study to report improved cognition following acute flavanol intake. Serial Threes accuracy was significant improved following both 520 mg and 994 mg CF compared to matched control drink, whilst the 994 mg CF drink produced quicker RVIP response times but more errors on the Serial Sevens.

Similarly, Field, Williams, and Butler (2011) carried out a randomized, single-blinded, counterbalanced, crossover study of 30 healthy adults, consuming dark chocolate (720 mg CF) and matched control white chocolate with 1-week washout between visits. Three visual tasks and two cognitive tasks were performed at baseline and 2 hours post consumption, the time point when peak blood flow is observed following cocoa flavanol ingestion (Francis, Head, Morris, and Macdonald (2006); D. J. Lamport et al. (2015)) . Here, there was significant improvement in visual contrast sensitivity and time taken to detect motion direction following the dark chocolate intervention. Furthermore, in comparison to white chocolate, ingestion of the dark chocolate significantly improved spatial memory and accuracy on the choice reaction time test. Further investigations of CF supplementation on cognitive performance have been conducted by Massee et al. (2015) who investigated both acute and sub-chronic effects of 250 mg CF. Cognitive performance was measured 2 hours after consumption, using 3 repetitions of a repeated 10 minute cycle of the cognitive demand battery which included a serial subtraction tasks (Serial Threes and Sevens) and a Rapid Visual information processing task . At the same time of CDB assessment, a mental fatigue scale was also measured. The findings showed that only performance on the Serial Sevens task in the first cycle of the CDB showed statistical significant after acute supplementation of 250 mg CF. No any other significant improvement of sub-chronic supplementation and was shown. Cardiovascular function was measured including cerebral blood flow, central blood pressure and peripheral blood pressure with no change at any measures.

To summarise, acute supplementation of flavonoid produces significant improvements in a number of different cognitive domains, with particularly significant effects seen 2 hr after consumption. This 2 hr time point is the point at which peak blood flow is observed following cocoa flavanol ingestion ((Francis et al. (2006); D. J. Lamport et al. (2015); Morris et al., 2006)) suggesting that changes in blood flow may be particularly important in mediating these acute actions. Again, it is difficult to draw comparisons between studies because of the differences in doses, treatments, cognitive tasks used, and study designs. Further investigations of acute flavonoids supplementation is needed to replicate and extend the evidence in support of the cognitive benefit seeming to arise from acute supplementation.

1.5.5 Evidence from supplementation studies in children

While there have been some limited studies investigating the effects of both acute and chronic supplementation of flavonoids in children, few well-controlled studies exist. Calderón-Garcidueñas et al. (2013) were the first to supplement children (mean age = 10.55 year old) with 680 mg of cocoa flavanols (30 gm of dark cocoa) daily for 9-24 days period. Fifteen of the eighteen participants performed significantly better on one or both of the cognitive tasks (letter and object span tests) post intervention. Unfortunately, not all of the participants completed the entire study, with the duration of intervention ranging from 9 to 24 days. Additionally, the letter and object span tests were performed within 4 hrs of the last dose on the final intervention day which means that the significant improvement in the memory performance may be effected by acute intake rather than chronic supplementation. Furthermore, sugar intakes were not controlled in the children and so, it is plausible that the cognitive boosts were due to greater sucrose intake in the children and not the flavonoids themselves.

Moving now to acute supplementation regimens, a study by Whyte and Williams (2015), included 14 children ages 8 to 10 year-olds who consumed either flavonoid-rich blueberry milkshake drink (143 mg of anthocyanins) or matched drink (matched for sugar and vitamin C) with 7 day washout between drink. The cognitive battery included the Go-NoGo, Stroop, Ray's Auditory Verbal Learning Task, Object Location task and a Visual N-back and was performed 2 hrs after the intervention drink. This study showed that in comparison to the matched drink, a flavonoid-rich blueberry drink produced a significant boost in delayed recall on the RAVLT. This study showed, for the first time, a boost in cognitive performance (memory performance) in school age children following acute flavonoid intervention. However, no significant improvement from acute flavonoid-rich blueberry intervention on the attention, response inhibition and visuospatial memory were observed.

In another study, Whyte et al. (2015) have recently investigated the acute effect of either 30 g wild blueberry drink (253 mg of anthocyanins), 15 g wild blueberry drink (127 mg of anthocyanins) or control drink on cognitive performance in 7 to 10 year old school children. The cognitive battery included a Auditory Verbal Learning Task (AVLT), Modified Flanker Task, Go-NoGo, and a Picture Matching Task (PMT) to ascertain effects of the intervention on word recognition, verbal memory, response interference, response inhibition and levels of processing. Participants performed the battery at baseline, 1.15 h, 3 h, and 6 h after treatment. There were significant improvements in final immediate recall of words at 1.15 h, better delayed word recognition at each time point, and finally, improved accuracy on incongruent trials in the flanker task at 3 h. Interestingly, the best cognitive performance was performed following 30 g wild blueberry drink and worst performance following the placebo drink. In term of memory performance, even though participants performed progressively worse throughout the test day, when compared to the control drink, both wild blueberry drinks showed significantly less decrements in performance across the test day.

1.6 Flavonoids and Mood

In term of the effects of food on mental health, a number of studies show positive associations between healthy food intake (basically in fruits and vegetable) and psychological well-being. In this section I will briefly review the studies of fruits and vegetable consumption, particularly flavonoids, and effects on mental health.

A cross-sectional study in Japan has demonstrated that consumption of green tea is associated with lower depressive symptoms in community-dwelling elderly Japanese (aged ≥70). In this study green tea intake over 1 month period was measured (by a self-administered questionnaire) and depressive symptoms (using a 30-item geriatric depression scale) were investigated. The results showed that the participant who consumed higher levels of green tea had lower prevalence of depression symptoms. Even after adjustment for the confounding effects of age, chronic disease, inflammatory status, cognitive impairment, disability, body mass index, lifestyle factors and psychological factors, the result remained significant showing that high frequency of green tea drink inversely associated with prevalence of depressive symptoms (Niu et al., 2009).

An epidemiology study conducted in 9255 church attendees in North America assessed the effects of a Mediterranean diet (as measured by a food frequency questionnaire) on mood (as measured by the Positive and Negative Affect Schedule). Here, there was a positive association between intake of a Mediterranean diet and better mood, which remained even after confounding factors including age, gender, ethnicity, body mass index, education level, sleep and sleep squared, exercise, total caloric intake, alcohol intake and time between the questionnaire were controlled. Specifically, vegetables, fruit, olive oil, nut and legumes were positively associated with better mental health, whilst sweets, desserts, soda and fast food frequency were associated with greater negative affect (Ford, Jaceldo-Siegl, Lee, Youngberg, & Tonstad, 2013).

A further cross sectional study investigating the association between fruit and vegetable intake and mental health status were conducted by the Canadian Health Survey (CCHS), which repeated a cross-sectional study of Canadians aged 12 years and older in five waves between 2000 to 2009. Frequency of consumption were used to assess fruit and vegetable intake and the Composite International Diagnostic Interview-Short form were used to measure occurrence of a major depressive episode over the previous 12 months as a major outcome. The result show that in the first wave, fruit and vegetable intake had a significant association with lower rates of depression, whilst once all data from all five waves were combined those individuals with the greatest intake of fruit and vegetable had lower levels of mental health issues. These consistent results were shown across other waves that poor mental status and occurrence of a previous mood and/or anxiety disorder were negative associated with fruit and vegetable consumption (McMartin, Jacka, & Colman, 2013).

Chang et al. (2016) conducted the cross-sectional study in nurses aged 36 to 80 year old without previous history of depression symptoms at the beginning of study. A validated food frequency questionnaire was assessed every 2 to 4 years to measure the consumption of total flavonoids and subclass of flavonoid (flavonols, flavones, flavanones, anthocyanins, flavan-3-ols, and polymeric flavonoids). A periodic self-reported questionnaire, history of antidepressant used, and depression diagnosed by physician or clinician were used to investigate depression. The incident of depression during the 10 years of study was shown to be inversely associated with flavonols, flavones and flavanones intake. In the older sample (age ≥ 65 at baseline or during follow up) there was a significant negative association between all of the flavonoids subgroups, except flavan-3-ols, and the symptoms of depression.

A number of intervention studies have also shown similar effects to the studies described above. Laura McMillan, Lauren Owen, Marni Kras, and Andrew Scholey (2011) studied the effects of dietary change on mood and cognitive function in healthy individuals. Twenty-five young adult women were randomised to either a ‘diet change’ intervention group (which provided an eating plan outlining the food to be consumed including fruits, vegetables, fatty fish, nuts and seeds, low fat natural dairy and wholegrain cereals) or a ‘no change’ control group who consumed their usual daily diet. Mood was measured using the 65-item Profile of Mood states (POMS) and Bond-Lader Visual Analogue Scales (VAS). The mood measurements showed significant improvements in self-reported vigour, alertness, and contentment in the ‘diet change’ group compared to the ‘no change’ group indicating that the diet rich in fruit and vegetable may associated with positive mood. Further support for the positive impact of fruit and vegetables on mental health was reported by J. Lee et al. (2015) who conducted a crossover design in 24 healthy woman (mean age 25.6), who were randomly allocated into either Mediterranean diet or habitual diet for a 10 days period and then switching to the alternate diet for the same duration. At the beginning and after completing each diet (days 11 and 22); mood, cognitive performance, blood pressure, blood flow velocity and arterial stiffness were measured. The Profile of Mood States (POMS) questionnaire was used to measure six dimensions of mood. The result of the study repeated the association between mental health and diet pattern from previous cross sectional studies (Ford et al., 2013; McMartin et al., 2013) and randomised controlled trial (Laura McMillan et al., 2011). There was statistically significant improvement in contentment and alertness, as well as a reduction in confusion for those participants on the Mediterranean style diet. There were also significant improvements in cognitive ability of switching and a significant reduction in cardiovascular measure of augmentation pressure. No any other associations were observed.

Moving beyond fruits and vegetables, there is evidence to support an association between cocoa flavonoids and mood (Massee et al. (2015)). Here they investigated the effect of acute and sub-acute effects of either 250 mg cocoa tablet supplementation or placebo tablet in young healthy Australians. Participants were tested between baseline and 2 hours later, as well as at baseline and after 4-weeks of daily consumption. Mood, mental fatigue and stress were measured by Cognitive Demand Battery (CDB), while cognitive performances were investigated by Computerized Cognitive Assessment Battery (SUCCAB). The secondary outcomes of blood pressure and changes in cerebral blood flow were also measured. Participants in the cocoa group showed significantly less mental fatigue prior to completing the CDB at the acute time point but not at the sub-chronic time point.

In summary, the limited available evidence with regard to flavonoids and mental health suggest that is a promising area, which need further studies to confirm whether flavonoid consumption influences mental health.

1.7 Overview of cognitive performance in existing flavonoids studies

A vast number of different cognitive tasks have been used to assess the effects of flavonoids on cognitive function. As can be seen from Table 1-3, acute and chronic RCT studies of flavonoids on cognition have utilised a wide variety of cognitive tasks with varying success. Executive function tasks typically include Serial Threes and Serial Sevens subtraction tasks, Continuous Performance Task (CPT), Digit vigilance, Stroop, Logical reasoning, Trail

Marking Test (TMT) A and B, Verbal Fluency Test (VFT), Letter memory and Go-NoGo. Attention is typically measured by serial subtraction tasks (Alharbi et al., 2015; Massee et al., 2015; Scholey et al., 2010) with only one study by Scholey et al. (2010) finding beneficial effects of flavonoids with a serial three subtraction task. The Continuous Performance Task (CPT) measures sustained and selective attention, has been used in one study and no significant effect was observed (Alharbi et al., 2015). Digit vigilance, which measures sustained attention, has thus far failed to demonstrated beneficial effects of flavonoid intervention (A. W. Watson et al., 2015). To measure inhibition, Stroop and Go-noGo have been employed but no positive effects have been shown (Crews Jr et al., 2008b; Kean et al., 2015; Massee et al., 2015; A. W. Watson et al., 2015). In terms of updating, TMT (Crews Jr et al., 2008b; Mastroiacovo et al., 2015) and Letter memory (Kean et al., 2015) have been used with only Mastroiacovo et al. (2015) finding positive effects on TMT after flavonoids intake. Verbal Fluency was also used in one study with positive effect was shown (Mastroiacovo et al., 2015).

In terms of memory tasks such as Immediate recognition, Spatial working memory, Contextual memory, delayed recognition, ModBent task, CERAD, Selective Reminding Test, Wechsler Memory Scale-III have all been employed. The spatial memory task was used by four studies (Camfield et al., 2012; Field et al., 2011; Massee et al., 2015; Pipingas et al., 2008) and two studies (Field et al., 2011; Pipingas et al., 2008) reported significant effects. The Immediate recall task was utilised by three studies (Alharbi et al., 2015; Massee et al., 2015; Pipingas et al., 2008) with only Pipingas et al. (2008) showing positive effect of flavonoids. Further memory task that shown positive effect of flavonoids was ModBent, which is a hippocampal-dependent memory task (Brickman et al., 2014). Other memory tasks used without no significant effect were Contextual memory, Delayed recognition, CERAD, Selective Reminding Test, and Wechsler Memory Scale-III.

Information processing speed tasks that have been used to investigate effects of flavonoids include the Simple and Complex Finger Tapping, DSST, Rapid visual information processing (RVIP), Choice reaction time, and Simple reaction time. Three tasks, to date, including Finger Tapping (Alharbi et al., 2015), RVIP (Scholey et al., 2010; A. W. Watson et al., 2015), and Choice reaction time (Field et al., 2011) appear to be sensitive to the effects of flavonoid intervention.

Overall, the overview of cognitive performance in existing flavonoids studies reviewed here are still unclear as to which cognitive domains and functions are sensitive to flavonoids supplementation.

Table 1-3 Cognitive tasks used in existing flavonoids studies

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
Acute supplementation studies					
Alharbi et al. (2015)	24 Males adult aged 30-65 (mean 51,sd 6.6)	Randomized, double-blinded, counterbalanced.	Subjects consumed 240 ml orange juice and a caloric-matched drink on 2	1. Immediate Word Recall. 2. Simple and Complex	Significant improve in the individual cognitive task including

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
			days separated by 2 weeks washout.	Finger Tapping. 3. DSST. 4. Continuous Performance Task (CPT). 5. Serial Sevens.	simple finger taping and Continuous Performance Task but no significant between 2 groups in combined cognitive score.
Bondonno et al. (2014)	6 males and 24 females adult (no age provided)	Randomized, controlled, cross-over trial.	Subjects were allocated in 4 condition. 1. Control. 2. Apple. 3. Spinach. 4. Apple +spinach.	Cognitive Drug Research battery.	No significant effect on cognition.
A. W. Watson et al. (2015)	36 adult aged 18-34 (mean	Randomized, double-blinded,	3 drink condition	1. Digit vigilance. 2. Stroop.	RVIP accuracy were

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
	24.8,sd 3.93)	placebo-controlled, cross-over trial.	with 1-week washout. 1. Control drink. 2. Anthocyanin blackcurrant extract 3. A cold-pressed blackcurrant fruit juice.	3. Rapid visual information processing (RVIP). 4. Logical reasoning.	improved after blackcurrant extract intake and increased reaction time of digit vigilance after blackcurrant juice intake. No significant difference in Stroop, logical reasoning
Scholey et al. (2010)	30 adult aged 18-34 (mean 21.9,sd 0.61)	Randomized, double-blinded, controlled, Balanced,	Subjects consumed 1. Control drink	1. Serial Threes 2. Serial Sevens	Serial Threes performance was significant improved

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
		tree period cross-over trial.	2. 520 mg cocoa flavonoid drink 3. 994 mg cocoa flavonoid drink by 3 days washout.	subtraction tasks 3. Rapid visual information processing (RVIP).	following both 520 mg and 994 mg CF compared to matched control drink. Only 994 mg CF drink was significant quicker in RVIP response time.
Field et al. (2011)	8 males and 22 females adult age 18-25 (no mean provided)	Randomized, single-blinded, controlled, order counterbalanced, cross-over trial.	Subjects consumed 1. Dark chocolate (720 mg CF) 2. Matched control white chocolate by	1. Visual spatial working memory. 2. Choice reaction time.	Dark chocolate improved spatial memory and ability on some aspect of the choice

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
			1 week washout.		reaction time test.
Massee et al. (2015)	40 adult aged 18-40 (mean 24.13,sd 4.47)	Randomized, double-blinded, placebo-controlled trial.	Same day and daily for four-weeks supplementation of placebo and 250 mg cocoa.	Swinburne University Computerized Cognitive Assessment Battery(SUCCAB) 1. Simple reaction time. 2. Choice reaction time. 3. Immediate recognition. 4. Congruent Stroop	No significant in SUCCAB. In CBD, Serial Sevens task in cycle one have shown statistic significant after acute supplementation of 250 mg

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
				colour word. 5. Incongruent Stroop colour word. 6. Spatial working memory 7. Contextual memory. 8. Delayed recognition. Cognitive demand Battery (CBD) 1. Serial Threes 2. Serial Sevens	

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
				subtraction tasks 3. Rapid visual information processing (RVIP).	
Chronic supplementation studies					
Mastroiacovo et al. (2015)	90 elderly (no age provided)	Double-blinded, controlled, parallel-arm trial.	8 week supplementation of 1. 993 mg Flavonol 2. 520 mg Flavonol 3. 48 mg Flavonol 2. Matched control white chocolate by 1 week washout.	1. Mini-Mental State Examination (MMSE). 2. Trial Marking Test (TMT) A and B. 3. Verbal Fluency Test (VFT).	HF and IF groups in comparison to LF have demonstrated a significant quicker to complete the TMT A and B. The performance was repeated

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
					in VFT score that the HF group perform greater than IF and LF.
Brickman et al. (2014)	37 adult aged 50-69 (no mean provided)	Randomized, controlled trial.	3 months consumed 1. 900 mg of cocoa flavanols. 2. 10 mg of cocoa flavanols.	Modified-Benton (ModBent task).	High flavanol condition shown a significant association to ModBent task.
Camfield et al. (2012)	63 adult aged 40-65 (no mean provided)	Randomized, double-blind placebo controlled trial.	30 days consumed 1. 500 mg of cocoa flavanols. 2. 250 mg of cocoa flavanols. 3. Placebo.	Spatial Working Memory	No difference effect on behavioural measures of accuracy and reaction time.

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
Kean et al. (2015)	37 adult aged 60-81 (mean 66.7,sd 5.3)	Crossover, double-blind randomized trial.	8 weeks supplementation and 4 weeks washout. 1. 305 mg of flavanone orange juice. 2. 37 mg of flavanone orange juice.	1. Go-NoGo. 2. DSST. 3. Letter memory. 4. CERAD.	Significant better in global cognitive function (mean of all test combined).
Pipingas et al. (2008)	42 males adult aged 50-65 (no mean provided)	Double-blind, control trial.	5 weeks supplementation of 1. 960 mg daily of Enzogenol® (Pinus radiate Bark Extract Formulation containing 80 % proanthocyan	1. Contextual memory. 2. Immediate recognition. 3. Simple reaction time. 4 Choice reaction time.	Significant quicker in response time for spatial working memory and immediate recognition after supplementation of 960

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
			-idins and other water soluble flavonoids). 2. 120 mg vitamin C control drink.	5. Visual vigilance. 6. Complex visual vigilance. 7. Spatial working memory. 8. Delayed recognition memory.	mg Enzogenol®.
Crews Jr et al. (2008b)	101 older adult age more than 60 year (no mean provided)	Double-blind, placebo-controlled, Fixed dose, parallel-group clinical trial.	6 weeks supplementation of 1. 37 grams dark chocolate bar. 2. Placebo.	1. Selective Reminding Test. 2. Wechsler Adult Intelligence Scale-III. 2. Wechsler Memory Scale-III. 3. TMT.	No significant effect were reported.

Authors	Subjects(n)	Design	Intervention	Cognitive tests	Key finding
				4. Stroop Colour-Word Test.	

1.8 Summary and Conclusion

Several important epidemiological, as well as acute and chronic intervention studies have shown positive effects of fruit and vegetable consumption on cognitive performance. Importantly, the flavonoids, found in high concentrations in fruit and vegetables, produce positive effects on cognition across all age groups and therefore represent a potential avenue worthy of further investigation.

Flavonoids, in particular fruit-based flavonoids, may produce effects on cognitive performance by enhancing vascular function, neuronal growth, gene expression, and cell signalling. However, acute and chronic supplementation of flavonoids have demonstrated beneficial effects on cognitive performance (memory and executive function), as well as mood and mental health. However, some inconsistency of effects on cognitive ability and mental performance have been observed, which require further investigation. These inconsistent results from flavonoid supplementation trials may arise from a number of potential confounds (listed below) and make comparison between the flavonoid studies very difficult:

1. the use of different foods and beverages as sources of flavonoids
2. the different dose of flavonoids used between studies
3. the cognitive tasks not being sensitive enough to detect rather subtle cognitive effects
4. the different age group of participants whereby flavonoids may show different effect at different ages
5. the different durations of the intervention themselves.

The beneficial outcome of flavonoids from evidence reviewed in this chapter have mainly employ adult and older adult populations, with limited studies in children. The potential benefits of supplementation to children, who are undergoing a period of rapid neurocognitive development, could be highly significant both in the short- and longer-terms. For example, administering an intervention which has known benefits to memory and attention could help children to concentrate in their lessons and learn more efficiently, boosting academic performance. Whilst over the longer-term, promoting healthy eating and providing a diet rich in flavonoids may benefit cognitive evolution decreasing the incidence of cognitive decline in the older adult population.

Thus, during my PhD, I will investigate the long-term effects of flavonoid-rich, readily available Thai fruits on cognitive performance in students aged 11-13.

Chapter 2 Summary of study design, aims and objectives

2.1 Introduction

The main aim of this thesis is to investigate the effects of long-term supplementation of flavonoid-rich fruits on the cognitive performance and mood of primary school aged children in Thailand. Positive cognitive effects from foods rich in flavonoids could be important in terms of influencing academic attainment in the setting of a school. Throughout this thesis, I will focus on the effects of long-term supplementation with flavonoid-rich fruits and their effects on both global cognitive performance as well as domain-specific aspects of cognition, such as executive function and episodic memory. Alongside this, I will investigate whether flavonoid rich foods can influence academic attainment by measuring changes in the O-Net score, a real-world indicator of academic performance, in Thai primary school students when they finish grade 6. Finally, I will assess changes in mood using the positive affect and negative affect scale (PANAS) before and after long-term flavonoid intervention in this age group. Overall, my thesis will be the first set of studies to consider the impact of long-term flavonoid-rich food and impact on real-life educational outcomes.

2.2 Justification for study design

2.2.1 Primary School-aged Participants

There are a number of reasons for selecting the participants used in this set of studies. All participants were in primary school grade 6 students (age 11-12 years old) in the Khonkaen municipality of the Royal Kingdom of Thailand. First of all, all Schools within this municipality provide the children with a lunchtime meal as part of the Thai School Lunch Programme (SLP). A typical SLP provides the midday meal in school, and is usually rice- or noodle-based with vegetables and a moderate amount of meat. After the main meal, a dessert is served, typically a fruit. By utilising this program, we can supplement high- or low-flavonoid fruits to our participants without any noticeable change in their normal food provision.

Secondly, it is well-documented that food habits during childhood have long term health effects and therefore, the promotion of healthy eating behaviour in children is crucial. Consumption of a healthy diet is key for optimal growth and development of children and teenagers, a diet rich in nutrients provides children with the opportunity to reach their maximal education potential (NHMRC, 2003), and eating a plentiful; a variety of foods during childhood establishes foods habits that will last into adulthood (Birch and Marlin, 1982). Thus, our fruit-based intervention will aid with the development of heathy eating behaviours in our participants.

Thirdly, growth of frontal lobes of the brain in children occurs in three stages contributing to developing cognitive functions: between birth and 5 years old, between 7 to 9 years old and between 11-13 years old (Anderson et al., 2004). The first spurt that occurs is between 0 to 5 years age when attentional control develops. The second spurt occurs between 7 to 9 years old and is involved in the development of cognitive flexibility, information processing and goal setting. Finally, the full maturation of executive control is developed in the third spurt between 11 to 13 years old (Spencer-Smith & Anderson, 2009). Thus, enrolling grade 6 students aged 11-12 years old will ensure that all children in the study have developed all the necessary cognitive skills to effectively attend to the tasks that were chosen in this thesis.

Finally, all students take the Ordinary National Educational Test (O-NET) at the end of grade 6 when they graduate (this is repeated in grade 9 and 12). The objectives of the O-NET are to: 1) test the knowledge and thinking ability of Grade 6 students according to the Basic Education Core Curriculum; 2) assess their academic proficiency according to the Basic Education Core Curriculum; 3) provide information to the schools to improve their teaching and learning activities and; 4) evaluate the quality of education at a national level. Eight subjects in the core curriculum are tested, that is, Thai language, Mathematics, Science, Social Studies, Religion and Culture, Health Study, Physical Education, Arts, Occupations and Technology, and Foreign languages. (http://www.niets.or.th/en/catalog/view/2211). Utilising outcomes from the O-NET test in Grade 6 students allowed me to assess the effect of a flavonoid-rich diet on real-life educational attainment.

2.2.2 Choice of fruits

In my study, I chose to supplement my participants with flavonoid-rich or flavonoid-poor whole fruits rather than offering the supplementation in the form of a daily tablet or capsule. A growing body of literature suggests that supplementation with whole foods (in this case, fruits) produce better health benefits than when using extracts (Keck, Qiao, & Jeffery, 2003; Mennen, Walker, Bennetau-Pelissero, & Scalbert, 2005). In addition, parents are likely to be more willing to allow their children to take part in a fruit-based intervention rather than an intervention that uses tablets or capsules.

During my interventions, a flavonoid-rich or flavonoid-poor fruit were incorporated into the SLP lunch menu. The choice of fruits used were dependent upon the season and availability in the local area. All fruits were prepared by school kitchen staff as part of their daily SLP duties.

Typically, the flavonoid-rich group consumed fruits such as pomelo (13,994.21 to 15,094.99 µg per 100 g fruit portion), guava (3,168.04 to 8,926.69 µg per 100 g fruit portion), pineapple (4,187.96 to 4,268.64 µg per 100 g fruit portion) over the course of the intervention which have been shown to be high in flavonoids (Kongkachuichai et al., 2010). The flavonoid-poor group consumed fruits such as watermelon (900 µg per 100 g fruit portion), mangosteen (1,515.18 µg per 100 g fruit portion), papaya (961 µg per 100 g fruit portion) and mangos (1,720 µg per 100 g fruit portion) which have been shown to be low in flavonoids (USDA, 2002); (Bhagwat, Haytowitz, & Holden, 2013).

In the context of Thai schools, teachers act as academic and health education providers to offer the students essential education, as well as good health. Thus, the teachers who are responsible for SLP played a role in encouraging my participants to consume the revised menus using instructions given to them. During the experimental period, the SLP teacher ensured that children were administered the correct flavonoid-rich or flavonoid-poor fruit, whilst the students themselves recorded their daily intake of the fruit using a food record I designed.

2.2.3 Cognitive domains and brain regions

The measurement of cognitive performance in my participants was an important consideration. The choice of cognitive task to detect the effects of a nutrition-based intervention needed to be free from floor- (too difficult for all participants) and ceiling-effects (too easy for all participants) and also needed to be sensitive enough to detect the changes in cognitive performance over the course of the intervention. Importantly, the tasks were selected to be sensitive to changes in specific aspects of cognitive performance that have previously been shown to be sensitive to flavonoid interventions. In the sections below, I will briefly describe the different cognitive domains and their associated brain regions that were targeted with my fruit intervention.

2.2.3.1 Attention

The term “attention” is the ability to focus and sustain attention to a particular stimulus and to divide attention between stimuli and distractors. Attention is particularly important for learning. The brain regions that underlie attention mature at difference stages of development. For instance, the ability to focus attention matures during mid-childhood, while the ability to sustain attention and inhibit distractions improves throughout adolescence and into early adulthood as the frontal lobes mature (Anderson, T., Manly, & Robertson, 1998).

2.2.3.2 Information processing

The information processing domain refers to the ability to rapidly and efficiently direct mental focus to relevant information from the environment and to make decisions appropriately and accurately. Integrity of neural communication within the central nervous system, as well as age-related development of neuronal myelination and number of neural connections in the central nervous system can influence speed of information processing. The speed of information processing is change during childhood but reverses in old age, which is an decrease in the time to process information when age increases (Kail, 1991, 2000). For example, Kail reported that the ability of 8 -10 year-old children to process information is 5-6 standard deviations lower than young adults, however this gap has closed to only 1 standard deviation by the time an individual is 12 - 13 years-old.

2.2.3.3 Memory

Attention, together with processing speed, influences learning and memory (Hughes & Bryan, 2003). The term of memory refers to the ability to retrieve information encoded verbally, visually and spatially. There are 3 features of memory consisting of short-term memory, working memory and long-term memory. Short term memory is the ability to store and retrieve information or stimuli within a period of approximately 30 seconds (Bryan, 1998; Lezak, 1995). Working memory, which is associated with the frontal lobes, is the ability to briefly store information, process and manipulate that information (Cohen, 1997). Long term memory refers to the ability to retrieval information after it has been stored for greater than 30 seconds, and this can take the form of facts (semantic memory), experiences (episodic memory), and motor skills.

2.2.3.4 Executive function

Executive function refers to complex and higher-order thinking processes, This domain is a cluster of abilities including planning (dealing with novelty to generate goals and decision making), switching or shifting (the ability to alternate between behaviour or information sources), updating (the ability to discard and replace information), inhibition (the ability to suppress automatic and habitual response or behaviours) and problem solving. The development of executive function is hypothesised to mature in late childhood after other cognitive process have developed across the brain (Baddeley, 1998; Leh, Petrides, & Strafella, 2010).

2.2.3.5 Global cognitive function

The term "Global cognitive function" refers to multiple domains of orientation, attention, memory, language, executive function and motor function. This term is used to represent overall cognitive performance, which taps into a variety of neural networks in different parts of the brain. This function is normally used to evaluate population or individual general cognitive status, often as a screening test. (de Jager et al., 2014).

2.2.4 Cognitive areas to be targeted with my interventions

As was described in chapter 1, acute and chronic supplementation of flavonoids to adult participants have been found to produce significant effects on memory (Field et al., 2011; Massee et al., 2015; Pipingas et al., 2008; Scholey et al., 2010), executive function (Mastroiacovo et al., 2015; A. W. Watson et al., 2015), processing speed (Alharbi et al., 2015; Field et al., 2011; Scholey et al., 2010; A. W. Watson et al., 2015), as well as global cognitive function (Kean et al., 2015). Much less literature is available for children, however flavonoid interventions in 7-10 year old children have shown positive effects on episodic memory and executive function (Whyte et al., 2015; Whyte & Williams, 2015). Beneficial effects of flavonoids on mental health has also been found in a number of studies (J. Lee et al., 2015; Massee et al., 2015; L. McMillan, L. Owen, M. Kras, & A. Scholey, 2011; Scholey et al., 2010). Given the positive association between flavonoid interventions, cognitive performance and mental health seen previously, my studies focussed on executive function, memory and mood.

2.2.5 Methodology

The literature investigating the cognitive benefits of flavonoids in children, though growing, is at present rather limited. This is especially so in relation to studies involving chronic supplementation to children. Given the paucity of literature in this area, my project will employ a long-term supplementation regimen to investigate the effects of flavonoid-rich fruits on cognition in children aged 11-13 years old.

A longitudinal human randomised controlled trial was employed with two conditions: high and low flavonoid supplementation in the Experiment 1 and Experiment 3, whilst three conditions were employed in the second experiment to include an additional control group, as well as high- and low-flavonoid conditions. In terms of the length of the intervention period, because the main outcome of the studies were the O-Net scores which were assessed at the end of academic year, a 12 weeks (or 1 semester) regimen was employed (7 weeks in Experiment 2 due to unanticipated difficulties in gaining access to the school). Cognitive ability and mood of all participants was tested at baseline and then repeated at the end of the supplementation period. Participants were asked to maintain their habitual diet outside of school for the duration of the studies. A range of cognitive tasks were used to assess a number of cognitive domains such as memory, executive function processing speed, global cognitive function and also mood, informed by previous studies that had shown sensitivity to flavonoids supplementation (Alharbi et al., 2015; Field et al., 2011; Kean et al., 2015; Massee et al., 2015; Mastroiacovo et al., 2015; Pipingas et al., 2008; Scholey et al., 2010; A. W. Watson et al., 2015) (Whyte et al., 2015; Whyte & Williams, 2015).

2.3 Aims of study

It is well-documented that flavonoid-rich foods can improve memory and learning performance in older and younger adults (Alharbi et al., 2015; Field et al., 2011; Kean et al., 2015; Massee et al., 2015; Mastroiacovo et al., 2015; Pipingas et al., 2008; Scholey et al., 2010; A. W. Watson et al., 2015) as well as children (Whyte et al., 2015; Whyte & Williams, 2015). However, these studies have not clarified the length of supplementation necessary to produce real-world changes in cognitive function (i.e. performance at school). The studies in my thesis attempts to address this issue.

The objectives of these studies are:

1. To examine the effect of flavonoid-rich fruits (commonly consumed in Thailand) on cognition performance and mood in school children aged 11-13 years old.
2. To examine the effect of flavonoid-rich fruits (commonly consumed in Thailand) on O-NET test performance in school children aged 11-13 years old.
3. To assess the length of flavonoid supplementation required to produce changes in cognition, mood and O-NET test performance.

Chapter 3 Effect of 12 weeks supplementation of flavonoid-rich fruits on memory, executive function, processing speed and mood in children aged 11-13 years old

3.1 Introduction

As detailed in Chapter 1, a number of studies have shown that the diet consumed by a child is critical for their cognitive development and performance, and is particularly influential on their academic achievement in school (Bellisle, 2004; Benton, 2010; Theodore et al., 2009). Typically, studies of dietary patterns and intelligence in early and middle childhood have shown associations between a child's cognitive development and the consumption of fish, breads and cereals, whilst consumption of margarine impairs cognitive functioning. In addition to these epidemiological studies, a number of intervention studies have also shown that cognitive performance can be influenced by intake of different nutrients. For example, 12 weeks supplementation with a commercially available multi-vitamin and mineral product (Pharmaton Kiddie) in 8-14 years old healthy children produces improvement in accuracy on attention tasks (Haskell et al., 2008). Whilst, Vazir and colleagues showed that 14 months supplementation with a micronutrient-fortified beverage in school aged children produced significant improvements in attention compared to a control group administered a placebo drink, although no changes in IQ, memory, or school achievement were seen (Vazir, Nagalla, Thangiah, Kamasamudram, & Bhattiprolu, 2006). As shown by Eilander et al. (2010) in a systematic review of multiple micronutrient supplementation in children, marginal increases in fluid intelligence and academic performance in healthy children can be seen following supplementation with such nutrients. Other intervention using only a single nutrient, such as zinc, iodine, iron (Eilander et al., 2010), and also anthocyanin, also show improvements in academic attainment and cognitive performance (Whyte et al., 2015; Whyte & Williams, 2015).

In recent years, the phytochemical constituents of particular foods and beverages have become a focus of investigation. As reviewed in Chapter 1, flavonoids, a class of compounds found in high concentration in a number of foods and beverages derived from Vitis vinifera (grape), Camellia sinensis (tea), Theobroma cacao (cocoa) and Vaccinium spp (blueberry), have shown reliable and reproducible beneficial effects on cognitive function. To date, a large body of evidence has emerged from human intervention studies demonstrating that the consumption of flavonoid-rich foods is associated with improvements in cognitive function (see reviews Williams & Spencer, 2012; Lamport et al. 2012; Macready et al, 2010). Although no chromic intervention studies in children have been published, acute flavonoid supplementation has been shown to produce beneficial effects on cognition in children. Here, children aged 7-10 show improvements in memory and executive function 2-6 hours post-administration (Whyte et al., 2015; Whyte & Williams, 2015). However, it is not clear from this research the age that we need to start flavonoid consumption, the dose range of flavonoids necessary to influence cognitive function or the particular aspects of cognitive function affected by flavonoid supplementation. Importantly, whether the proven short-term effects of flavonoid supplementation result in improved academic performance across a school year has also not been investigated.

Thus, in my first study I will investigate the effect of 12 weeks supplementation with a diet enriched with a high flavonoid fruit, or matched low flavonoid fruit, on cognitive performance and academic achievement. Importantly, this will be the first study to consider the impact of long-term supplementation and the impact on real-life educational outcomes.

3.2 Methods

3.2.1 Participants

179 primary school grade 6 students (age 11-12 years old) from Wat Klang Municipal school, Khonkaen province (Thailand) were recruited for this study. The students were recruited from 4 classes, each containing 44-46 students. Within each class, 50% of the children were randomly allocated to the control (low flavonoid, LF) group and the remaining 50% were allocated to the experimental (high flavonoid, HF) group (n=~22-23 per group for each class). In total, 88 students were allocated to the HF group and 91 students to the LF group. Table 3-2 show the demographic and characteristic of remaining participants.

3.2.2 Intervention Fruits

The Thai School Lunch Programme (SLP) is a nationwide scheme that provides all children with their midday meal in school. The meal is usually rice-based with vegetables and a moderate amount of meat or a noodle soup with vegetables and meat. During the 12-week intervention, children additionally received a flavonoid-rich (HF group) or flavonoid-poor (LF group) fruit (approximately 40-50 grams) after their main meal. The choice of fruit depended upon the season and availability in the local area. Typically, the flavonoid-rich group consumed fruits such as pomelo, guava, pineapple and orange over the course of the intervention, whilst the flavonoid-poor group consumed fruits such as watermelon, melon, papaya and banana (USDA, 2007). The fruits were administered daily by the class teachers, and all children recorded their own daily intake of the intervention. The content of flavonoids in fruits show in the Table 3-1 (see below).

Table 3-1 Flavonoid content of the supplied intervention fruits

Group	Fruit	Flavonoids (µg) per 100g whole fruit	Flavonoids((µg) per 40g portion
HF	-Pomelo	13,994.21 to 15,094.99	5817.84
	-Guava	3,168.04 to 8,926.69	2418.99
	-Pineapple	4,187.96 to 4,268.64	1691.32
	-Orange	44320	17728
LF	-Watermelon	900	244
	-Melon	640	256
	-Papaya	961	485.14
	-Banana	3,560.53 to 4,063.6369	1524.83

3.2.3 Cognitive Tests

The Auditory-Verbal Learning Task and Digit Symbol Substitution Tasks were completed using pen and paper, all other tasks were performed using E-Prime V 2 running on a PC to display the task and record the responses.

3.2.3.1 Auditory-Verbal Learning Task (AVLT)

The AVLT is a task commonly used to measure learning, and memory recall of words. The English version of the task was adapted for use in Thailand by creating 4 lists of 15 Thai words. Thai word lists were created for each test session (administered randomly across the test sessions) and were matched for concreteness and familiarity. Briefly, at each test session, participants performed five consecutive free recalls (Recalls 1 to 5) of the same 15 nouns (List A) presented auditorily at a rate of 1 word per second. A further list of fifteen nouns (List B) was then presented as an interference list and recalled once only (Recall B). There was then a further free recall of List A (Recall 6) followed by a fifteen minute delay and then a final free recall of List A (Recall 7).

For each test session the following measures were calculated: (i) Word span- the number of words recalled on recall 1; (ii) Final acquisition- the number of words recalled on recall 5; (iii) Total acquisition- the total number of words recalled on the first 5 trials; (iv) Pro-active interference- the number of words on recall 1 minus the number of words recalled on the interference list; (v) Retroactive interference- the number of words on recall 5 minus the number of words on recall 6; (vi) Delayed recall- the number of words recalled on recall 7.

3.2.3.2 Digit Symbol Substitution Task (DSST)

The DSST measures response processing speed, attention, visuo-spatial skills and shifting skills and has been employed previously for use in school aged children (Pradhan & Nagendra, 2009; Van der Elst, Dekker, Hurks, & Jolles, 2012).The test requires the participant to complete as many unique geometric symbols with their corresponding Arabic numbers within a time limit of 90 seconds. As can be seen in Figure 3.1, the digit symbol codes were given at the top of the paper and 118 empty squares which were randomly numbered 1-9 for participants to complete with the corresponding symbol. In this version of the task, participants were asked to complete as many of the boxes as they could with the symbol corresponding to the number given. Participants were requested to start in the first row, working in sequence from left to right, without missing out any of the squares. Participants were given 90 seconds for this task. Therefore, participants were scored for each of the item correctly completed. Two alternate versions of this task were employed and randomly assigned for test either pre-test or post-test.

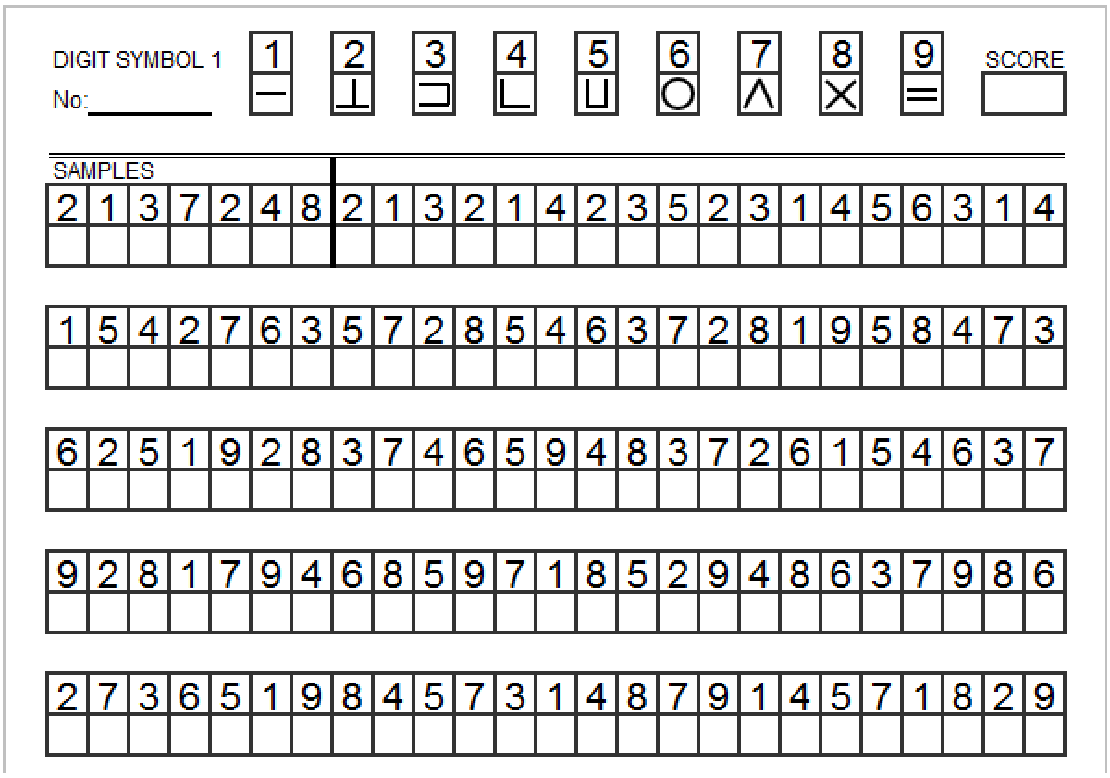

Figure 3-1 Example of DSST

3.2.3.3 Letter Memory Task

The letter memory task investigates working memory. Here, on each trial participants were presented with a series of random letters (one letter presented at a time) from the 44 letters in the Thai alphabet (Figure 3-2, left panel). Participants were instructed to remember the last four letters presented on the screen (updating as each new letter appeared). At the end of the letter presentation phase, 4 sets of 4 letters appeared (Figure 3-2, right panel) and participants were instructed to indicate which set of 4 letters represented the last 4 letters that had appeared in the sequence. Participants then had 6 seconds to indicate their choice by selecting on the keyboard which colour label corresponded with the correct response. There were 18 trials in the test with 6 easy trials comprised of a sequence of 5 letters, 6 medium trials containing 7 letters and 6 hard trials containing 9 letters. Two different versions of the task were produced and randomly assigned for presentation either pre-test or post-test. The mean score of overall response time and accuracy of the task for each participant were calculated individually.

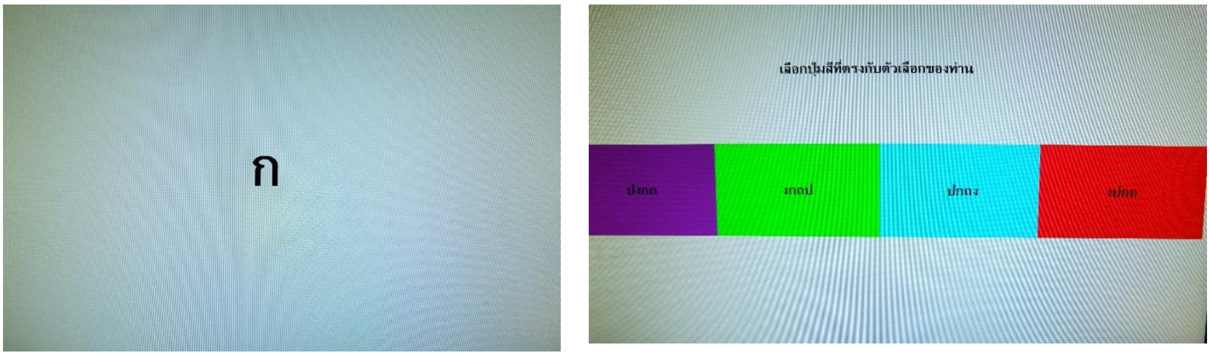

Figure 3-2 Example of letter memory with left panel presented a series of random letters and right panel presented a set of 4 letters represented the last 4 sequence letters.

3.2.3.4 Flanker Task

The Flanker test is used to measure participant's attention and inhibitory control. Arrow symbols were presented five in a row in the centre of computer screen. This task required a participant to respond to the direction of the central arrow of the set of five by pressing the corresponding arrow key on the computer keyboard as quickly as they could. Two types of trial were administered: in congruent trials, the direction of the central arrow was identical to the pairs of flanking arrows on either side of it (<<<<< or >>>>>), whereas in incongruent trials, the central arrow was in the opposite direction to the flanking arrows on either side of it (<<><< or >><>>). The participants were instructed to press the right or left arrow key as fast as they could be corresponding to the direction that the middle arrow was pointing. Each trial was shown for 120 ms followed by 1000, 1300 or 1500 ms pseudorandom inter-stimulus interval.

Response times and accuracy for congruent and incongruent trails were measured individually. The interference effect was also calculated by subtracting the mean response time of incongruent trials from congruent trials.

3.2.4 Other measures

3.2.4.1 O-Net test

At the end of the academic year, as described in Chapter 2 all grade 6 children completed the Thai Ordinary National Educational Test (O-NET). The O-Net test was marked independently by a public organisation, NIETS (National institute of Educational Testing Service (Public organization). Overall scores and individual subject-specific scores for each pupil were returned to the school and were used in my study as a measure of academic attainment.

3.2.4.2 Positive and Negative Affect Scale (PANAS)

The PANAS, developed by Watson, Clark and Tellegen (D. Watson & Clark, 1988)), is a 20 item self –report checklist to investigate positive (e.g. interested, excited, pround) and negative affect (e.g. distressed, upset, guilty). The 10 positive items reflect an individual experiences pleasurable engagement with the environment, whereas the 10 negative items indicate subjective distress and un-pleasurable engagement. A paper and pencil Thai language version of the task was created using the same descriptors in the English language version.

For each item, at baseline and post-intervention, participants were asked to complete the PANAS assessing their mood by writing down the number that correlated to how they felt at the present moment on a 5-point Likert scale (1= very slightly/not at all to 5= extremely). The scores for the 10 words relating to positive emotions, and 10 words relating to negative emotions, were added together to give an overall measure of a participant's positive affect (PA) and negative affect (NA). An example of the PNS can be found in Appendix C.

3.2.4.3 Semi-quantitative Food frequency questionnaire

The first part of the questionnaire asked participants to complete their demographic status such as age, gender, number of family members, family income, height and weight. Additionally, participants were asked whether they regularly consumed any food supplements (i.e. multi-vitamin supplements).

In the second part of the questionnaire, typical food consumption was assessed using a food frequency questionnaire (FFQ). This FFQ was adapted for use in Thailand by the researcher and included reference to typical foods found in Thailand. The FFQ consisted of 23 items of food, which consisted of 3 categories of carbohydrate, 10 categories of protein, 5 categories of fruit and vegetables, and 5 categories of snack (a copy of the FFQ can be found in Appendix D). The FFQ was completed 1 week before the experiment started, a week in the middle of the intervention phase and then during the last week of the intervention. Analysis of these diaries allowed us to check the basic diets of participants and to note any changes in food selection over the course of the experiment.

3.2.4.4 Daily food record

Students were also asked to keep a daily food record which documented all food consumed during the SLP. In the daily record students were instructed to indicate how much food they had consumed (consumed in total, partially eaten and not eaten at all) for both the main meal and the intervention fruit. Analysis of these data allowed us to calculate consumption of the flavonoid-rich or flavonoid-poor fruit daily (see Appendix E).

3.2.5 Procedure

On each test day, the school teacher allowed me to test participants out of their normal classroom during morning time, participants were seated in a classroom to test the pre-test in order as below:

AVLT trials 1-5, interference trial and trial 6 took approximately 10 minutes.

DSST test took approximately 2 minutes.

Positive and negative affect schedule (PANAS) approximately 5 minutes.

Self-report semi quantitative FFQ took approximately 15 minutes.

Delay recall of RVLT took approximately 1 minute.

In terms of the computer-based cognitive test, flanker and letter memory took approximately 3 and 10 minutes respectively on the day after pencil and paper tests. The participants were out of normal classroom to complete the computer base tasks for approximately 20 minutes and came back to classroom after they had finished.

During 12 weeks of the intervention period students consumed their habitual meals and were given the intervention fruits by class teacher in school lunchtime. The participants were also asked to keep a daily food record which was collected weekly throughout the length of intervention.

The intervention ended by the last Friday of January (week 12 of the intervention) and the participants completed the O-Net score by Saturday. The cognitive tests were tested in the same order as at baseline in the week after the end of intervention.

3.3 Analysis

3.3.1 Data Clean-Up

Of the 179 participants recruited to the study, participants that consumed less than 50% of the intervention fruit across the 12 weeks of the study, or completed less than 75% of the cognitive testing over the course of the study, were excluded from the final analysis. In total, 80 participants from the HF group and 81 participants from the LF group remained in the analysis.

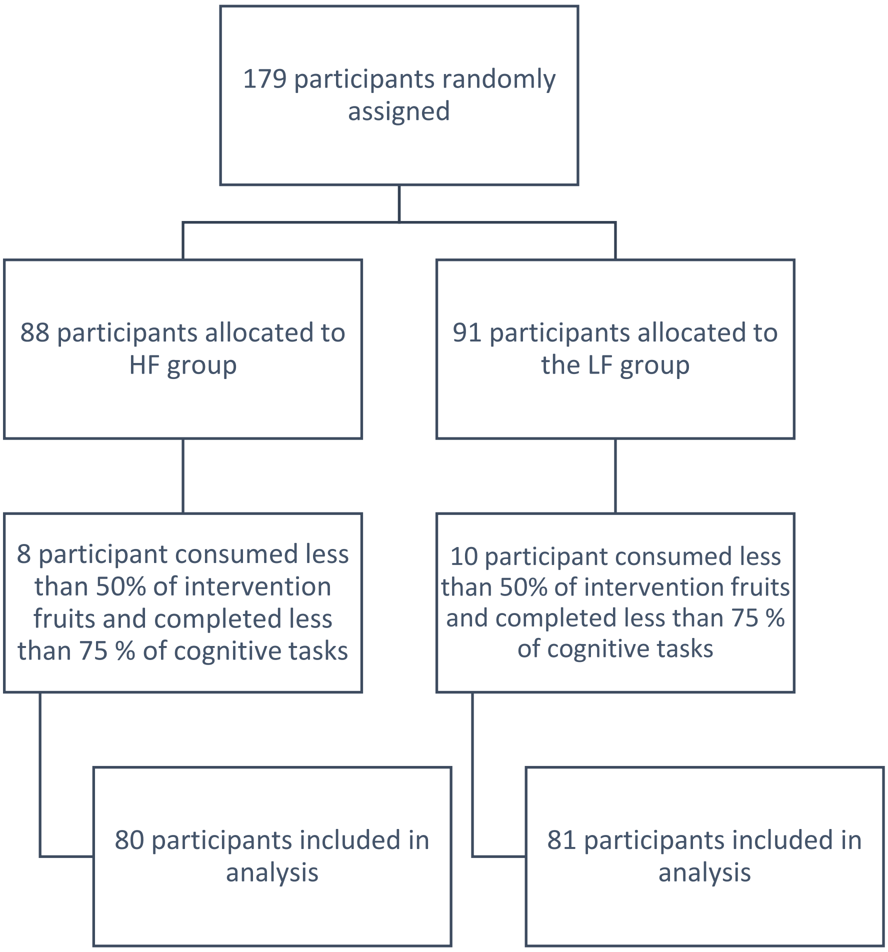

Figure 3-3 Diagram of data clean-up

3.3.2 Intention to treat (ITT) analysis

As described by Armijo-Olivo et al (2009), ITT has become the “gold standard” for clinical RCTs maintaining the advantage of treatment randomisation whilst accounting for non-compliance and participant withdrawal (Armijo-Olivo, Warren, & Magee, 2009). In my study, the ITT technique was adopted for analysis of the cognitive and mood data.

3.3.3 FFQ

For each food type, the number of portions consumed was multiplied by the frequency of consumption per week. This value was then divided by 7 to calculate the average number of portions consumed per day.

3.3.4 Flavonoids content from intervention fruits

Flavonoids content from the intervention fruits, which have come from USDA data (orange, papaya, water melon, melon and banana) and the Thai publication (guava, pineapple and pomelo), was calculated from the daily food record. The record of how much of the intervention was consumed (1 = consumed in total, 0.5 = partially eaten, 0 =not eaten at all) was then multiplied by the flavonoid content of the intervention (i.e. per 40g portion) on that particular day. This was repeated for all days of the intervention period, added together and divided by the number of days in the intervention period to average flavonoid intake per day.

3.3.5 High and low scores

In general, higher scores and positive scores of each variable indicate better performance. In contrast, for letter memory response time, flanker task interference effect and also the composite score of executive function, lower scores and negative increases over time indicate better performance.

3.3.6 O-NET performance

The 8 subjects of O-Net score including Thai language, Mathematics, Science, Social Studies, Religion and Culture, Health Study, Physical Education, Arts, Occupations and Technology, and Foreign languages were marked independently by NIETS (National institute of Educational Testing Service to yield a percentage score. The individual subject-specific scores for each participant were returned to the school and were used in my study as a measure of academic attainment by generating a proportion score for each subject.

3.3.7 Individual task performance

The DSST was scored for each participant by correctly completing in each symbols with the corresponding number, with higher scores indicating better psychomotor processing.

In term of AVLT scores 2 categories of the score were calculated for each participants including total acquisition which is a number of total words of the first 5^{th} trials and delayed recall which is the number of words recall in trial 7^{th}.

For the letter memory task and flanker task, the mean accuracy and response time were calculated for each subject for pre and post-intervention. The accuracy of correct responses was calculated as a percentage, whilst mean response times were calculated as the average response time in milliseconds for all correct responses.

3.3.8 Composite cognitive score

To create the composite scores for memory and executive function the raw scores for performance on individual tasks performance were converted to z scores. The memory composite score consisted of the average z score of total acquisition and delay recall measures from the RAVLT while the executive function composite score consisted of the average z score of letter memory response time and the interference effect of the flanker task.

3.3.9 Statistical Analysis Plan

SPSS version 22.0.0 was used to analyse the data of this thesis. A 2x2 Repeated measures Analysis of variance (ANOVA) with 2 flavonoid treatments (2 groups: HF or LF) and 2 time points (2 time points: pre- and post-intervention) were used to detect the effect of flavonoids between treatments and over time changes for each of the cognitive tasks. This was followed, where appropriate, by Bonferroni comparisons. Changes in O-NET score were assessed by independent T-test.

3.4 Results

3.4.1 Demographics

Descriptive statistics for baseline demographic characteristic of participant are shown in Table 3-2. The ages of participants ranged between 11 to 13 years old, with a mean age of 12.07 (SD=0. 47). There were 88 males (HF=46; LF=42) and 73 females (HF=34; LF=39). Weight ranged from 27 to 71 kgs, with a mean of 45.09 (SD=9.93), being higher, although non-significantly, in the HF group (M=45. 71, SD=10.16) compared to the LH group (M=44.52, SD=9.74). Height of participants ranged from 130 to 171 cms (M=151.81, SD=8.21), with HF group (M=151.45, SD=8.01) being slightly shortly lower than LF group (M=152.13, SD=8.42). No difference reached significance.

Family income in HF group was more than 30,000 Thai Baht per month (27.50%) and LF group was 5,001 to 10,000 Thai Baht per month (24.69%). Number of family members ranged from 2 to 15 people, with 5.12 (SD=1. 88) in HF group and 4.95 SD=1.66) in LF. Grade point average of grade 5 ranged from 1.30 to 4.00 (M=2.86, SD=0.79), slightly higher in LF group (M=2.91, SD= 0.80) compared to the HF group (M=2.81, SD=0.78).

There were no statistically significant differences in socioeconomic status between HF and LF participants at baseline.

Table 3-2 Baseline demographic characteristic of participants

Baseline Characteristics	HF Mean (SD) (n =80)	LF Mean (SD) (n =81)	Total Min (n =161)	Total Max (n =161)	Total Mean (SD) (n =161)
Age (years of age)	12.01(0.47)	12.07(0.47)	11	13	12.04(0.47)
Gender:					
Females	34	39	-	-	76
Males	46	42	-	-	103
Anthropometrics:					
Weight (kg)	45.71(10.16)	44.52(9.74)	27	71	45.09(9.93)

Baseline Characteristics	HF Mean (SD) (n =80)	LF Mean (SD) (n =81)	Total Min (n =161)	Total Max (n =161)	Total Mean (SD) (n =161)
Height (cm)	151.45(8.01)	152.13(8.42)	130	171	151.81(8.21)
% Family income (Thai Baht /Month)					
0-5,000	12.50	7.41	-	-	9.94
5,001-10,000	16.25	23.46	-	-	19.88
10,001-15,000	17.50	24.69	-	-	21.12
15,001-20,000	10.00	14.81	-	-	12.42
20,001-25,000	3.75	4.94	-	-	4.35
25,001-30,000	7.50	8.64	-	-	8.07
More than 30,000	27.50	14.81	-	-	21.12

Baseline Characteristics	HF Mean (SD) (n =80)	LF Mean (SD) (n =81)	Total Min (n =161)	Total Max (n =161)	Total Mean (SD) (n =161)
Number of family members	5.14(1.93)	4.95(1.66)	2	15	5.03(1.77)
Grade Point Average From Grade 5	2.81(0.77)	2.91(0.80)	1.30	4.00	2.86(0.79)

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; TB/M= Thai Baht per month.

3.4.2 Daily Food record and Food Frequency Questionnaire

As can be seen in Figure 3-4, the estimated average total amount of flavonoids consumed from the intervention fruits was 6,245.15 µg/day (SD=956.34) for the HF participants, and 227.80 µg/day (SD=49.55) in the LF group.

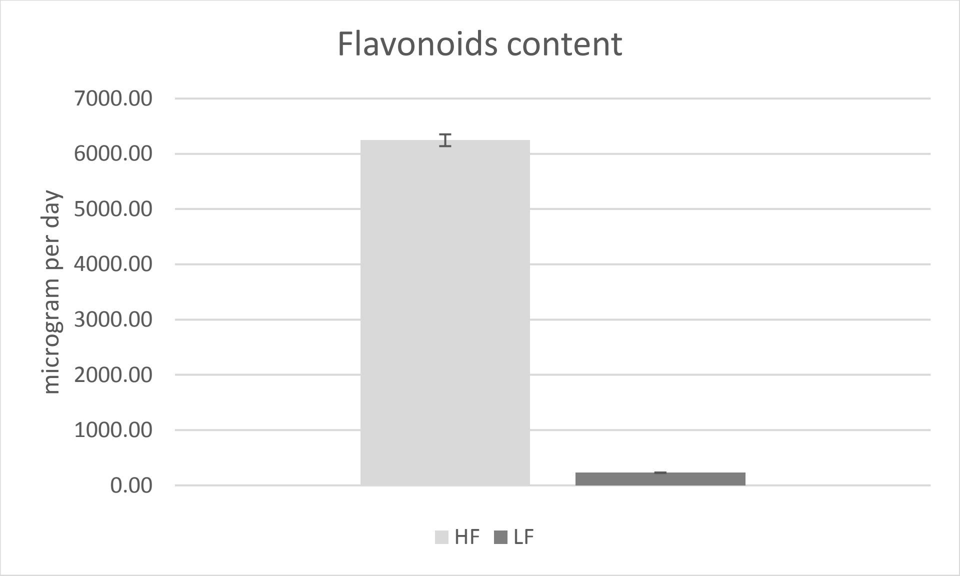

Figure 3-4 Flavonoid content per day from intervention fruits during intervention period

Data from the Food Frequency Questionnaire revealed that the average numbers of the food portion consumed for each food group as shown in Table 3-3. The independent t-test revealed that there was a statistical difference in FFQ 1 (baseline) in some food groups with higher intake in HF participants than LF participants including fruit (HF=4.04; LF=3.19) (t (159) = 2.413, p =.017) and Juice (HF=1.32; LF=0.86) (t (159) = 2.110, p =.036). In FFQ 2 the significant difference between 2 group with HF participants still higher intake than in LF participant in meat (HF=7.53; LF=5.91) (t (159) = 2.062, p =.041) and soft drinks (HF=1.42; LF=1.00) (t (159) = 2.027, p =.044). There were no significant differences between groups in food intake in FFQ3.

As can be seen in Table 3-4, despite our randomisation procedure, fruit and vegetable consumption (including the intervention fruit) in HF participants was significant higher than in LF participants (F (1,159) =4.051, p =.046). However, no significant difference between groups were seen when analysed separately for fruit (F (1,159) =3.173, p =.077) or vegetable intake (F (1,159) =2.311, p =.076) despite the HF group consuming slightly more portions of both fruit and vegetables a day.

Table 3-3 Portion of food consumption from Food Frequency Questionnaire of participants

		HF Mean(SD)	LF Mean(SD)	*t*	*p*
Carbohydrate	*Carbohydrate*				
	1stFFQ	6.72(4.23)	7.51 (4.32)	-1.170	.243
	2ndFFQ	6.39(4.19)	6.11(4.68)	0.398	.691
	3rdFFQ	5.62(4.21)	5.10 (3.79)	0.822	.412
Meat and Protein	Meat				
	1stFFQ	7.61(5.74)	6.45(4.56)	1.418	.158
	2ndFFQ	7.53(5.38)	5.91(4.53)	2.062	.041*
	3rdFFQ	6.06(4.67)	6.10(4.70)	-0.051	.959
	Milk				
	1stFFQ	1.90(1.22)	2.09(2.02)	-0.740	.460
	2ndFFQ	1.98(1.52)	1.92(1.69)	0.247	.805
	3rdFFQ	2.08(2.12)	1.93(1.84)	0.488	.627

		HF Mean(SD)	LF Mean(SD)	*t*	*p*
	Soy				
	1stFFQ	1.38(1.45)	1.40(2.06)	-0.057	.954
	2ndFFQ	1.26(1.42)	1.08(1.38)	0.812	.418
	3rdFFQ	1.21(1.59)	1.06(0.87)	0.727	.468
	Egg and Bean				
	1stFFQ	3.00(2.38)	2.69(2.10)	0.875	.383
	2ndFFQ	2.34(1.41)	2.19 (1.94)	0.583	.561
	3rdFFQ	2.15(1.69)	2.05(1.55)	0.409	.683
Fruit and Vegetable					
	Vegetable				
	1stFFQ	3.35(2.31)	3.29(2.41)	0.169	.866
	2ndFFQ	3.34(2.58)	2.71(1.76)	1.804	.073
	3rdFFQ	3.12(2.43)	2.57(2.26)	1.483	.140

		HF Mean(SD)	LF Mean(SD)	*t*	*p*
Fruit	1stFFQ	4.04(2.46)	3.19(1.98)	2.413	.017*
	2ndFFQ	3.97(2.86)	3.90(3.05)	0.145	.885
	3rdFFQ	4.37 (3.78)	3.51(2.64)	1.667	098
Juice and snack	Juice				
	1stFFQ	1.32(1.58)	0.86(1.17)	2.110	.036*
	2ndFFQ	1.04(1.32)	0.89(1.11)	0.763	.447
	3rdFFQ	0.82(0.75)	0.90(0.93)	-0.661	.510
	Snack and Dessert				
	1stFFQ	2.60(2.53)	2.73(1.91)	-0.369	.713
	2ndFFQ	2.51(1.82)	2.37(1.74)	0.501	.617
	3rdFFQ	2.37(1.75)	1.99(1.66)	1.416	.159

	HF Mean(SD)	LF Mean(SD)	*t*	*p*
Soft Drink				
1stFFQ	1.81(1.94)	1.31(1.63)	1.770	.079
2ndFFQ	1.42(1.40)	1.00(1.17)	2.027	.044*
3rdFFQ	1.26(1.23)	1.04(1.07)	1.238	.218
Candy				
1stFFQ	1.46(1.92)	1.23(1.82)	0.788	.432
2ndFFQ	0.68(0.95)	0.70(1.06)	-0.128	.899
3rdFFQ	0.72(0.98)	0.52(0.56)	1.620	.107

Table 3-4 Portions of fruit and vegetable, individually and combined, consumed per day during the course of the intervention.

Group	FFQ 1 Mean(portion) Sd	FFQ 2 Mean(portion) Sd	FFQ 3 Mean(portion) Sd	*F*	*p*
F&V					
1(HF)	7.39(3.82)	7.31(4.42)	7.49(5.45)	T:0.131	.878
2(LF)	6.48(3.30)	6.61(3.68)	6.08(3.92)	G:4.051	.046*
				TXG:0.490	.601
V					
1(HF)	3.35(2.31)	3.34(2.58)	3.12(2.43)	T:2.601	.076
2(LF)	3.29(2.41)	2.71(1.76)	2.57(2.26)	G:2.311	.130
				TXG:1.070	.343
F					
1(HF)	4.04(2.46)	3.97(2.86)	4.37(3.78)	T:0.981	.376
2(LF)	3.19(1.98)	3.90(3.05)	3.51(2.64)	G:3.173	.077
				TXG:1.497	.223

3.4.3 O-NET Score

An independent samples T - test was performed on total O-NET score

As can be seen in Figure 3-5, no significant differences in O-NET performance were found between HF and LF participants *t* (159) = -0.869, *p* =.386.

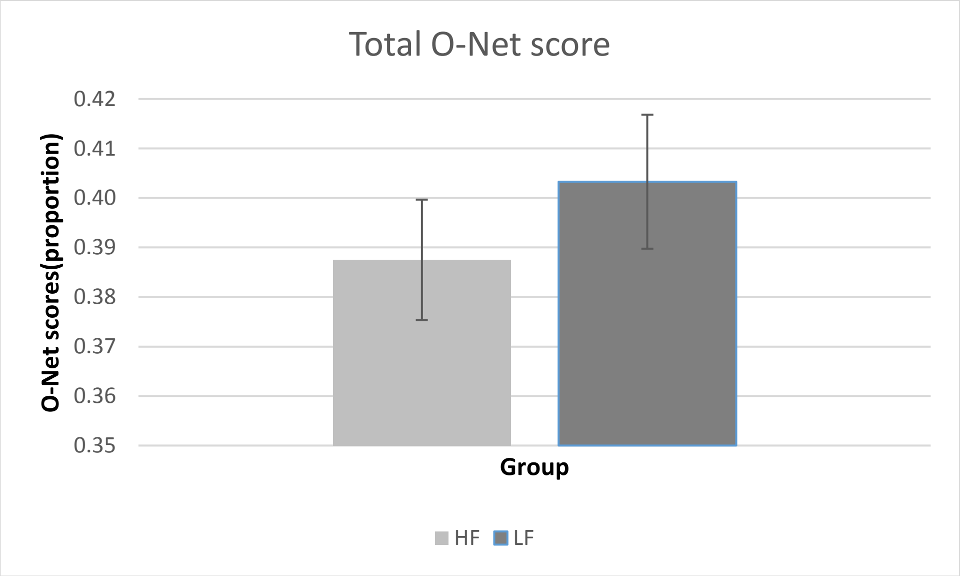

Figure 3-5 Mean total O-NET score (proportion score).

3.4.4 Composite score of Memory and Executive function

As can be seen in Figure 3-6 memory performance for both groups became worse over the course of the intervention time (F (1,159) =17.206, p =.000), however there were no significant differences between groups (F (1,159) =0.007, p =.931) or any group x time interaction (F (1,159) =3.445, p =.065). In terms of executive function as can be seen in Figure 3-7, the HF group showed better performance than LF at the end of intervention (-0.14 versus -0.01) but this failed to reach significance (F (1,159) =0.036, p =.849). However, there was a significant main effect of time (F (1,159) =4.144, p =.043) and a group x time interaction (F (1,159) =5.265, p =.023).

An independent samples T - test was performed to confirm the significant effect on executive function but this fail to reach significant in post intervention (t (159) = -1.070, p =.290).

3.4.5 PANAS Mood

For positive affect (PA), there were no significant group (F (1,159) = 0.056, p = .814), time (F (1,159) = 1.483, p = .225) or group x time effects (F (1,159) = 0.111, p = .739) during the experiment. In contrast, negative affect (NA) showed a significant time x group interaction (F (1,159) =5.265, p =.023), whereby at baseline, the HF group showed higher NA than the LF group (23.22 versus 22.19) but by the end of the intervention this pattern had reversed with the HF group having lower NA scores than the LF group (22.21 versus 23.19). There was no statistically significant main effect of time (F (1,159) = 0.000, p = .989) or group (F (1,159) = 0.001, p = .974). Furthermore, simple independent t-test was performed separately on NA, but also failed to reach significant difference (t (159) = -0.955, p =.341).

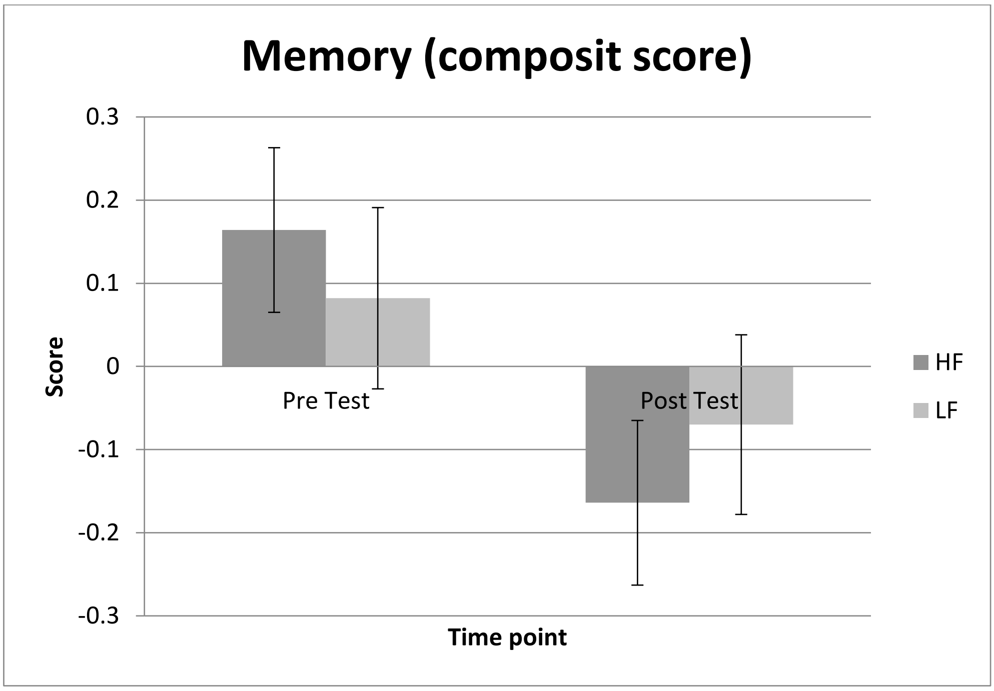

Figure 3-6 Composite score of memory (±standard error of the mean)

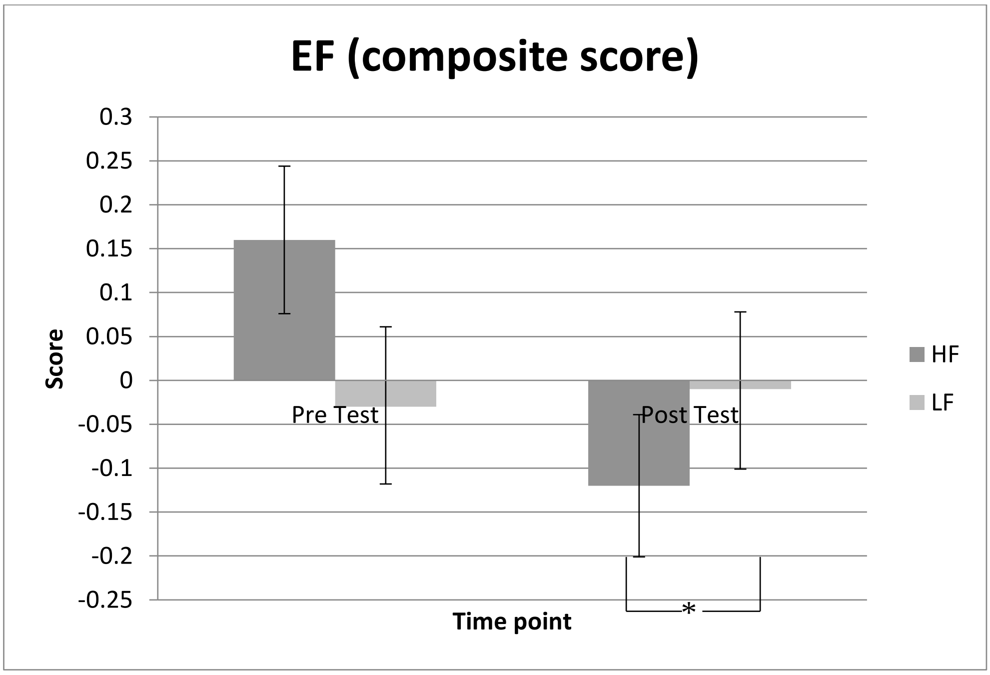

Figure 3-7 Composite score of Executive function (±standard error of the mean)

Table 3-5 Mood Measures: Z score, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all participants

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
PANAS	Baseline	33.03(5.96)	33.38(6.24)	T:1.483	.225
Positive affect	Post-Intervention	32.63(6.55)	32.69(6.17)	G:0.056	.814
				TXG:0.111	.739
Negative affect	Baseline	23.22(7.09)	22.19(5.92)	T:0.000	.989
	Post-Intervention	22.21(6.76)	23.19(6.20)	G:0.001	.974
				TXG:5.265	.023*

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	 *p*
O-Net Score		0.386(0.109)	0.403(0.122)	T (159) =-0.869	.386

3.4.6 Individual result of Individual cognitive task/mood

Table 3-6Table 3-1 (below) shows the mean individual performances on the RAVLT, DSST, letter memory, and flanker task for both HF and LF groups.

3.4.6.1 AVLT

In this task our primary measures were total acquisition and delayed recall, both measures that were used to calculate composite memory score. Total acquisition (total number of words learned across the first 5 recalls) showed HF performing better than LF at both baseline and post intervention (baseline: 40.08 versus 37.71 and post-intervention: 38.18 versus 37.27). However, there was no significant effect of time (F (1,159) = 1.662, p =.199), treatment (F (1,159) = 0.656, p = .419) or time x treatment interaction (F (1,159) = 0.633, p = .428). For delayed recall, there was a significant time x group interaction (F (1,159) = 5.628, p = .019) whereby HF performed better at baseline than LF (9.78 versus 9.47), while post intervention both groups decreased but less so for LF group (7.22 versus 8.41). As expected, there was a significant main effect of time (F (1,159) = 33.229, p = .000), but no significant effect of group (F (1,159) = 0.447, p = .505).

3.4.6.2 DSST

Both groups performed better post-intervention compared to at baseline, as reflected by a significant main effect of time (F (1,159) =72.394, p=.001). Although participants in the HF group scored better on the digit symbol substitution task at baseline (52.66 versus 50.86), as well as slightly better at post intervention (60.13 versus 59.43), than LF participants there were no significant effects of either treatment (F (1,159) = 0.583, p = .446) or time x group interaction (F (1,159) = 0.336, p = .563).

3.4.6.3 Letter Memory

The two groups performed the letter memory task with similar reaction times at baseline (HF group: 2823 ms and LF group: 2803 ms), while the HF participants performed better. However, there was no significant time x group interaction (F (1,159) = 1.282, p = .178). Both groups performed the task faster at the end of intervention compared to their performance at baseline resulting in a significant main effect of time (F (1,159) =14.146, p=.001). No difference between the groups was evident though (F (1,159) = 0.546, p = .461).

3.4.6.4 Flanker Task

Assessing the mean interference effect (calculated by subtracting incongruent RT from congruent RT) showed that LF participants performed better at baseline (HF group: 28.29 ms versus LF group: 11.68 ms) but slower post intervention (HF group: 23.55 ms versus LF group: 25.27 ms) than HF participants, repeated-measures ANOVA revealed a significant time x group interaction (F (1,159) = 4.109, p =.044). However, no significant main effects of time (F (1,159) = 0.959, p = .329) or group (F (1,159) = 1.017, p = .315) were apparent. Simple main effects analysis of the interference effect (t-test) showed no significant difference between the groups (t (159) = -0.196, p =.845).

A number of cognitive measures were obtained in the study such as word span, final acquisition, pro-active interference, retroactive interference from AVLT and the accuracy score for the executive measure were analysed but report separately in appendix J.

Table 3-6 Cognitive and Mood Measures: Mean, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all participants

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
Memory Function:					
RAVLT					
Total Acquisition	Baseline	40.08(14.14)	37.71(14.00)	T:1.662	.199
	Post-Intervention	38.18(13.55)	37.27(14.56)	G:0.656	.419
				TXG:0.633	.428

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
Delayed Recall	Baseline	9.78(4.15)	9.47(4.38)	T:33.229	.000*
	Post-Intervention	7.22(4.94)	8.41(4.92)	G:0.447	.505
				TXG:5.628	.019*
Executive Function:					
Letter Memory Task	Baseline	2823(653.26)	2803(696.61)	T:14.146	.000*
Letter RT	Post-Intervention	2489(732.44)	2646(779.76)	G:0.546	.461
				TXG:1.828	.178

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
Flanker Task	Baseline				
Flanker Interference	Post-Intervention	28.29(46.99)	11.68(60.97)	T:0.959	.329
Effect					
		23.55(41.53)	25.27(66.20)	G:1.017	.315
				TXG:4.109	.044*
Processing Speed:	Baseline	52.66(11.66)	50.86(11.83)	T:72.394	.000*
DSST	Post-Intervention	60.13(12.16)	59.43(11.90)	G:0.583	.446
				TXG:0.336	.563

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; T= Time; G= Flavonoids treatment group; TXG= time x Flavonoids treatment group interaction. *p<.05.

3.5 Discussion

This is the first study to investigate the effect of 12-weeks high flavonoid supplementation on cognitive performance and academic outcomes in grade 6 Thai school children. Although no significant differences in academic performance were seen, as assessed by performance on the end of year O-NET test, those children supplemented with a high flavonoid fruit did show better executive function (composite score) and lower negative mood than those children supplemented with a low flavonoid fruit. In contrast, no significant changes in composite memory performance or performance on the individual cognitive tasks were seen.

In terms of composite score for cognitive performance, we failed to demonstrate a beneficial effect of HF supplementation on overall memory performance. However, our study did show that students administered a HF fruit each day showed a significant improvement in composite score for executive function. Although no studies using chronic supplementation of flavonoids to children have been published, these results are somewhat in agreement with previous studies in adults. Firstly, Ye and colleagues showed that high consumption of fruit and vegetable can improve MMSE score and individual cognitive domains (including executive function, memory and attention) in middle age and older adults (Ye et al., 2013). Furthermore, Kean found the improvement in global cognitive function in healthy older adults flowing orange juice rich in flavonone consumption compared to those on low flavonone drink (Kean et al., 2015). Finally, in a study considering dietary intake of berries, those participants who consume greater quantities of berries, and therefore have a higher intake of flavonoids and anthocyanidins, showed a reduced rate of cognitive decline (Devore et al., 2012).

We also found a positive association between high flavonoid fruit consumption and lower negative mood. Here, the HF students demonstrated a lower negative affect score post intervention compared to those students supplemented with a LF fruit. These data are in agreement with numerous studies that have shown that a traditional Mediterranean diet (rich in flavonoid-rich fruit and vegetables) are beneficial for mental health. Although limited studies have been completed using children as a sample, a review by O’Neil and colleagues showed that children and adolescent who consumed higher quality of foods (healthy and nutritionally-dense foods) had better mental health than those who consumed lower quality or unhealthy foods (O’Neil et al., 2014). Furthermore, Lee found the improvement in contentment and alertness, as well as a reduction in confusion in healthy woman adult flowing Mediterranean diet (rich in fruit and vegetable) consumption compared to those on habitual diet (J. Lee et al., 2015). Similarly, Jacka and colleagues have shown that women (aged between 20-93) with higher fruit and vegetable intake over a 12 month period show a low risk of depression, dysthymias and anxiety disorders compared to those women who ate a typical Western diet with low fruit and vegetable intakes (Jacka et al., 2010). In addition, the effect on negative affect in our study is also consistent with a national survey of Canadians that showed an inverse association between higher fruit and vegetable consumption with lower odds of depression (McMartin et al., 2013). The study of Chang et al. (2016) also found the incident of depression during the 10 years of study that shown to be inversely associated with flavonols, flavones and flavanones intake. In the older sample (age ≥ 65 at baseline or during follow up), there was a significant negative association between all of the flavonoids subgroups, except flavan-3-ols, and the symptoms of depression. The negative association between fruit and vegetable consumption has also been confirmed by Richard, Rohrmann, Vandeleur, Mohler-Kuo, and Eichholzer (2015) who found that samples with higher intake of 5 a day fruit and vegetable had lower psychological distress than samples with lower intakes. Other study that support the positive effect of fruit and vegetable intake and mood was Ford et al. (2013) who found that high intake of fruit, vegetable, olive oil, nuts, and legumes had association with positive affect, whereas high intake of sweet/deserts, soda and fast food had an association with negative affect.

Disappointingly, we failed to show any effect on O-NET test performance between our two groups following the intervention. This finding may be explained by the fact that O-NET score depends on the complex, combined activities of the school to prepare the students for the test – the flavonoid fruit intervention itself may not have been long enough or the flavonoid content not high enough to further affect performance.

There a number of limitations to this study that need to be considered and may help explain the lack of significant beneficial effects of our HF intervention on the individual cognitive tasks. Firstly, the testing was completed in a small room adjacent to the main classrooms and it was difficult to maintain a quiet atmosphere for the duration of the test sessions. Furthermore, the length of test battery (~30-40 minutes per child) was perhaps too long for the children to fully maintain their concentration. Finally, there were a number of confounding factors that may have influenced the study (e.g., like or dislike intervention fruits, exchanging fruit with their friends) and although we attempted to control these throughout the intervention period, it is plausible that they occurred and may have adversely influenced the outcome of this study.

In summary, this study is the first study to examine the effects of 12 weeks supplementation with a flavonoid-rich or flavonoid-poor fruit on cognitive performance and academic achievement in primary school students. These result shows that consumption of high flavonoid fruit may have produced a positive effect on composite executive function (although not composite memory performance), but this did not translate to overall better academic performance. In addition, lower negative affect was seen in those children supplemented with high-flavonoid fruit. In my next study, I will further investigate the effect of high-flavonoid fruit on executive function and mood.

Chapter 4 Effect of 7 weeks supplementation of flavonoid-rich fruits on executive function, and mood in children aged 11-13 years old

4.1 Introduction

In Chapter 3, the main findings were a positive association between a composite score for executive function with flavonoid rich fruit intake, as well as a negative association with a measure of negative affect. During the 12 weeks of the study, the intervention participants consumed 40 grams per day of rich flavonoids fruits (Pomelo, Orange, Guava and Pineapple) while control participants consumed 40 grams of low flavonoids fruits (Water melon, Melon, Banana and Papaya). The rich flavonoid group demonstrated better executive function performance and reduced negative affect relative to low flavonoids participants.

These results add to the growing evidence base that high consumption of fruit based flavonoid or other flavonoid types have positive effects on cognition and mood. For example, Kean et al. (2015) conducted an 8 week crossover, double-blind randomized trial with 4 weeks washout between drink conditions. The participants were 37 healthy older adult (average age 67) whose diets were supplemented with a high flavanone 100 % orange juice containing 305 mg flavanone or a low flavanone orange-flavored cordial containing 37 mg of flavanones. Cognitive performance was measured by the Go-NoGo, DSST and the letter memory to evaluate executive function and the CERAD to evaluate episodic memory. The results demonstrated that the high flavanone condition performed better in global cognitive function (mean of all tests combined) comparison to the low flavanone condition. In terms of mood, Laura McMillan et al. (2011) studied the effects of dietary change on mood and cognitive in health individuals in a randomised controlled trial involving 25 young woman adults. The intervention group consumed a diet including fruits, vegetables, fatty fish, nuts and seeds, low fat natural dairy and wholegrain cereals, whereas a no change control group maintained their usual daily diet. Mood measurements showed significant improvements in self-related vigour, alertness, and contentment.

Experiment 1 (Chapter 3) showed better executive function following rich flavonoids fruit supplementation. Executive function in Chapter 3 was investigated using a flanker task and a letter memory task. In terms of individual tasks, only the interference effect of the flanker task showed a significant difference between the two conditions but not the letter memory task.

The next logical step therefore was to employ more executive function tasks to try to better understand the nature and extent of the executive function improvement. Therefore, this chapter reports a more detailed investigation of a flavonoid rich fruit intervention on a battery of executive function tasks in primary school children. In addition to a Flanker task and letter memory task, this study also employed a StopGo task to evaluate executive function. A previous study demonstrated the effect of high flavanone 100 % orange juice containing 305 mg flavanone versus low flavanone orange-flavored cordial containing 37 mg flavanone on cognitive performance by using the Go-NoGo , DSST and the letter memory (Kean et al., 2015). The StopGo and Go-NoGo task have both been used to measure response inhibition, with the former being employed in the current study in an attempt to replicate the effects of flavonoids on this executive function domain. However, in contrast to Chapter 3, we decided to allocate into three condition consisting of a proper control group (no supplementation of flavonoids), a low flavonoids group (LF, poor flavonoids fruits supplementation) and a High flavonoids group (HF, high flavonoids fruits supplementation). Doing this may allow us to clarify whether different levels of flavonoids intake or indeed fruit generally affect cognitive ability, particularly with respect to executive function.

The average quantity of flavonoids in the previous study was estimated to be 6,245.15 µg in HF and 227.80 µg in LF per day (from a 40 grams fruit portion). This is noticeably much lower in comparison to other studies where content has ranged from 250 mg to 994 mg of flavonoids (Camfield et al., 2012; Kean et al., 2015; Mastroiacovo et al., 2015; Pipingas et al., 2008). Whilst dose response profiles for flavonoids are still poorly understood, in the current study we decided to double the portion size such that participants were asked to consume 80 grams of fruits during each school lunch time. The variety of intervention fruits were the same as the Experiment 1 and reflected those that were seasonally available at a local market. As in the last experiment, it was expected that the high flavonoid condition would lead to better executive function and mood compared to the flow flavonoid and control condition.

Thus, this experiment employed a between subject randomized control trial design. The participants were tested at baseline and post intervention to evaluate whether 80 grams supplementation of rich flavonoids fruits during an 8 weeks intervention had positive effects on academic outcomes, executive function and mood. The presence of a non-fruit control condition would provide the opportunity to assess the benefits (if any) of additional dietary fruits to the diet independent of flavonoid content. Fruits contain other compounds (e.g. vitamin C, vitamin E) that may also have effects on cognition and other measures of wellbeing.

4.2 Methods

4.2.1 Participants

The subjects were 136 primary school grade 6 students (age 11-13 years old) from Wat Klang Municipal school in the 2014 academic year, Khonkaen province, Thailand. The students were split across 3 classes, each containing 44-46 students. For our experiment, children were randomly allocated to a control group (no flavonoids fruit, low flavonoid (LF) group and a high flavonoid (HF) group (n=~15-16 per group for each class). In total, 42 students were allocated to the control group, 47 students to the HF group and 47 students to the LF group. Participants that were judged to have consumed less than 50 percent of the intervention fruits were excluded from the statistical analysis. Furthermore, participants that completed less than 75 percent of the cognitive tasks in total across both measurement points also removed from the analysis. This left a total of 35 participants in the control group, 44 in the HF group and 43 participants in the LF group. Table 4-1shows the demographic and other key characteristics of the remaining participants.

4.2.2 Intervention Fruits

As explained in Experiment 1 the Thai School Lunch Programme (SLP) is a nationwide scheme that provides all children with their midday meal in school. The meal is usually rice-based with vegetables and a moderate amount of meat or a noodle soup with vegetables and meat. During Experiment 1, children additionally received a flavonoid-rich or flavonoid-poor fruit (approximately 40-50 grams) after this main meal. In this experiment, therefore we investigated the effects of rich flavonoids, low flavonoids and also no flavonoids by supplementation of 80 grams of intervention fruit. The flavonoid-rich group consumed fruits such as pomelo, guava, pineapple and orange over the course of the intervention and the flavonoid-poor group consumed fruits such as watermelon, melon, papaya and banana (USDA, 2007). Finally, the control group consumed a bun or biscuit (70-80 calories). The fruit interventions were administered daily by the class teachers, and all children recorded their daily intake of the intervention. The content of flavonoids in fruits show in Chapter 3.

4.2.3 Cognitive Tests

All cognitive tasks were performed using E-Prime V 2 running on a PC to display the task and record the responses.

4.2.3.1 Letter Memory

The letter memory task investigates working memory, as described in Chapter 3 section 3.2.3.3. Again, in this experiment, two different versions of the task were produced and randomly assigned for presentation either pre-test or post-test. The mean score of overall response time and accuracy of the task for each participant were calculated individually.

4.2.3.2 A modified Flanker Task

The Flanker test used to measure participant's attention and inhibitory control. The task in this chapter was adapted from Whyte (2015), started with an initial fixation point on the computer screen for 2000 ms. Following the fixation, either 4 types of cue (Figure 4-1 A) including a centre cue, a double cue, a spatial cue or no cue randomly appeared for 120 ms. Between the cue and the target, the fixation was presented in the centre of the screen for 400 ms. The target arrow symbols were presented below or above the fixation point in the centre of computer screen designating either a low load (single arrow), medium load (one row of five arrows) or a high load (two row of five arrows) (Figure 4-1 C) This task required a participant to respond to the direction of arrow by pressing the corresponding arrow key on the computer keyboard as quick as they could. Three types of trial were administered consisting of a neutral trial with a single arrow, congruent trials in which the direction of the central arrow was identical to the arrow on either side of it (<<<<< or >>>>>), and incongruent trials where the central arrow was in the opposite direction to the flanking arrows on either side of it (<<><< or >><>>). The participants were instructed to press the right or left arrow key as fast as they could correspond within 1000 ms to the direction that the middle arrow was pointing.

Response Times for congruent and incongruent trails were measured individually. We also calculated the interference effect by subtracting response time of incongruent trials ability from congruent trials ability.

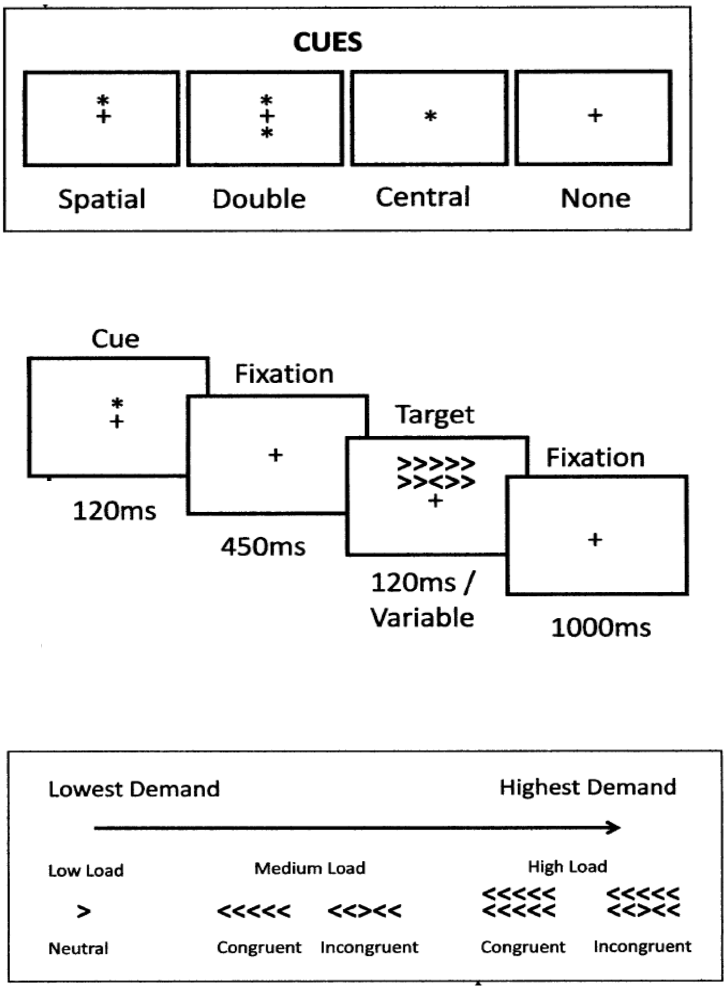

Figure 4-1 The diagrams of modified flanker task (Whyte, 2015).

4.2.3.3 StopGo task

A StopGo task was used to measure response inhibition. A child orientated version of the task was used which initially consisted of a fixation point depicting an empty hole that was shown on a computer screen for 700 ms which was followed by a stimulus slide of a cartoon mole popping from the hole for a duration of 1000 ms. Cartoon moles were either facing left or right and participants were asked to click a mouse button corresponding to the mole facing direction. One in four of the trials was the stop signal, consisting of a cartoon mole with a helmet on its head, which appeared after the delay. For the stop signal, participants were asked to stop themselves from clicking the mouse button. The objective of the game was introduced to the participants as trying to save the garden from cartoon moles stealing vegetables by click either left or right mouse button to "bash" him when he appeared and avoid bashing him when he appeared with a helmet. This task was started by a practice trial and the word "oops" appeared when participants failed to inhibit by clicking the mouse bottom in stop signal trials. The main trial included a single block of 200 trials in total (150 of go trial and 50 of inhibition trials) with no feedback of oops word for incorrect response.

Go trial reaction time and accuracy were measured for each participant.

4.2.4 Other measures

4.2.4.1 O-Net test

At the end of the academic year, as described in Chapter 2 all grade 6 children completed the Thai Ordinary National Educational Test (O-NET). The O-Net test was marked independently by a public organisation, NIETS (National institute of Educational Testing Service (Public organization). Overall scores and individual subject-specific scores for each pupil were returned to the school and were used in my study as a measure of academic attainment.

4.2.4.2 Positive and Negative Affect Scale (PANAS)

The PANAS, developed by Watson, Clark and Tellegen (D. Watson & Clark, 1988)), is a 20 item self –report checklist to investigate positive (e.g. interested, excited, pround) and negative affect (e.g. distressed, upset, guilty). The 10 positive items reflect pleasurable experiences with the environment, whereas the 10 negative items indicate subjective distress and un-pleasurable engagement. A paper and pencil Thai language version of the task was created using the same descriptors in the English language version.

For each item, at baseline and post-intervention, participants were asked to complete the PANAS assessing their mood by writing down the number that correlated to how they felt at the present moment on a 5-point Likert scale (1= very slightly/not at all to 5= extremely). The scores for the 10 words relating to positive emotions, and 10 words relating to negative emotions, were added together to give an overall measure of a participant's positive affect (PA) and negative affect (NA). An example of the PNS can be found in Appendix C.

4.2.4.3 Semi-quantitative Food frequency questionnaire

The first part of the questionnaire asked participants to complete some demographic questions. The demographics status questions included age, gender, a number of family members, family income, height, weight and details of any functional foods/supplements used.

Food consumption, the second part of the questionnaire, was assessed by using a food frequency questionnaire, developed by the researcher including a variety of fruits and vegetables. The FFQ consisted of 23 items of food, which assessed 3 categories of carbohydrate, 10 categories of protein, 5 categories of fruits and vegetables, and 5 category of snack. The frequency of consumption ranged from one per week to daily consumed per week. The FFQ was completed 1 week before the experiment started, a week during the middle of the intervention phase and then in the last week of the intervention. Analysis of the FFQ enabled assessment of the habitual diets of participants, and to note any changes in food choices/behaviour over the course of the experiment (see Appendix D).

4.2.4.4 Daily record

Students were also asked to keep a daily food record to record food consumed during the school lunch programme (SLP). In the daily record students were instructed to indicate the appropriate response that reflected their eating (consumed in total, partially eaten and not eaten at all) for their main meal and intervention fruits. Analysis of these data enabled us to calculate approximate consumption of the flavonoid-rich or flavonoid-poor fruit daily (see Appendix E).

4.2.5 Procedure

Participants were introduced to general information about the study, its purpose and objective via a letter to all parents. They were also asked to complete a consent form and brought the parent/guardian consent form to their parent/guardian then brought back via school teacher after signed.

On each test day, school teacher allowed me to test participants out of their normal classroom during morning time, participants were seated in a classroom to test the pre-test in order as below:

> Positive and negative affect schedule (PANAS) approximately 5 minutes.
>
> Self-report semi quantitative FFQ took approximately 15 minutes.

In term of the computer based cognitive test the letter memory, flanker task and stop-go task respectively took approximately 20 minutes in the day after paper base measure. The participants were out of normal classroom to completed computer base tasks for approximately 30 minutes and came back to classroom after they had finished.

During 7 weeks of the intervention period students consumed habitual meals and were given the intervention fruits by class teacher in school lunchtime. The participants were also asked to keep a daily food record which was collected weekly throughout the length of intervention.

The intervention ended by the last Friday of January (week 7th of intervention) and the participants completed the O-Net score by Saturday. The cognitive tests were tested in the same order as at baseline in the week after the end of intervention.

4.3 Analysis

4.3.1 Data Clean-Up

Of the 136 participants recruited to the study, 14 participants failed to consume at least 50% of their intervention across the 7 weeks of the trial and were subsequently excluded from the analyses. In total, data from 35 control participants, 44 HF participants and 43 LF participants were analysed. The diagram of participant allocation is shown in Figure 4-2.

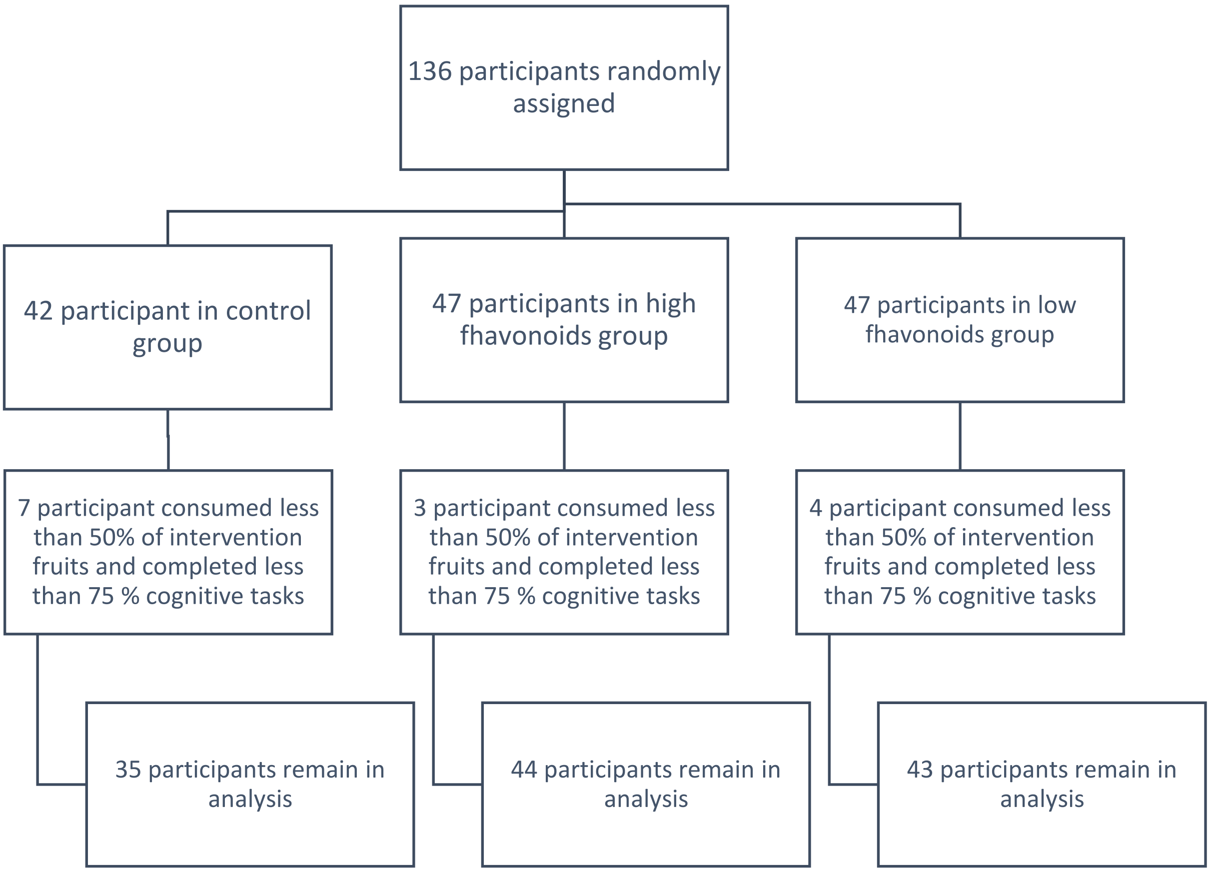

Figure 4-2 Diagram of data clean-up

4.3.2 FFQ

For each food type, the number of portions consumed was multiplied by the frequency of consumption per week. This value was then divided by 7 to calculate the average number of portions consumed per day.

4.3.3 Flavonoids content from intervention fruits

Flavonoids content from the intervention fruits, which have come from USDA data (orange, papaya, water melon, melon and banana) and the Thai publication (guava, pineapple and pomelo), was calculated from the daily food record. The record of how much of the intervention was consumed (1 = consumed in total, 0.5 = partially eaten, 0 =not eaten at all) was then multiplied by the flavonoid content of the intervention (i.e. per 40g portion) on that particular day. This was repeated for all days of the intervention period, added together and divided by the number of days in the intervention period to average flavonoid intake per day. In this study the flavonoids content in HF group was 13,257.70 µg per day and in LF was 1,032.83 µg per day.

4.3.4 High and low scores

In general, higher scores and positive scores on each variable indicate better performance. In contrast, for letter memory response time, flanker task interference effect and also the composite scores of executive function, lower scores and negative increases over time indicate better performance.

4.3.5 O-NET performance

The 8 subjects of O-Net score including Thai language, Mathematics, Science, Social Studies, Religion and Culture, Health Study, Physical Education, Arts, Occupations and Technology, and Foreign languages were marked independently by NIETS (National institute of Educational Testing Service to yield a percentage score. The individual subject-specific scores for each participant were returned to the school and were used in my study as a measure of academic attainment by generating proportion score for each subject.

4.3.6 Individual task performance

For the letter memory task, flanker task, and StopGo, the mean accuracy and response time were calculated for each subject for pre and post-intervention. The accuracy of correct responses was calculated as a percentage, whilst mean response times were calculated as the average response time in milliseconds for all correct responses.

4.3.7 Composite cognitive score

To create the composite scores for memory and executive function the raw scores for performance on individual tasks were converted to z scores. The composite score of executive function, we calculated an overall z-score based on the interference effect of the flanker task, average response time for the letter memory, and response time for the stop-go task that represented the primary measures for each of these tasks.

4.3.8 Statistical Analysis Plan

SPSS version 22.0.0 was used to analysis the data of this thesis. A 3x2 Repeated Measures Analysis of variance (ANOVA) with 3 flavonoid treatments (3 groups: Control, HF and LF) and 2 time points (2 time points: pre- and post-intervention) were used to detect the effect of flavonoids between treatments and over time changes for each of the cognitive tasks. This was followed, where appropriate, by Bonferroni comparisons. O-NET scores was measured and analysed by independent One-Way ANOVA.

4.4 Results

4.4.1 Demographics

Descriptive statistics for baseline demographic characteristic of participant are shown in Table 4-1.The age of participants at the time of the study was between 11 to 13 years old, with a mean age of 11.96 (SD=0.33). There were 64 males (Control=19, HF=17; LF=28) and 58 females (Control=24, HF=18; LF=16). Weight ranged from 26 to 85 kgs, with a mean of 46.66(SD=12.33), being slightly higher in the HF group (M=47. 58, SD=10.67) than LH (M=47.44, SD=11.79) and control group (M=44.97, SD=14.09). Height of participants ranged from 128 to 170 cms (M=153.39, SD=8.72), with HF group (M=154.41, SD=8.02) being slightly shorter than the LF group (M=154.79, SD=8.15) but taller than the control group (M=151.21, SD=9.52)

The high percent of family income in both group were 5,000 to 10,000 TB per month (HF=22.86%, LF =38.46% and control =41.86). Number of family members ranged from 2 to 13 people, with 4.91 (SD=1.50) in HF group and 4.70 SD=2.13) in LF and 5.26 in control (SD+1.95). Grade point average of grade 5 ranged from 1.47 to 4.00 (M=3.09, SD=0.69), with a numerically higher GPA in the HF group (M=3.17, SD= 0.80) than control (M=3.10, SD=0.64) or LF (M=3.00, SD=0.76) group respectively.

There was no statistically significant of socioeconomic status between HF and LF participants at baseline.

Table 4-1 Baseline demographic characteristic of participants.

Baseline Characteristic	Control (SD) (n=42)	HF Mean (SD) (n =47)	LF Mean (SD) (n =47)	Total Min (n =136)	Total Max (n =122)	Total Mean (SD) (n =122)
Age (years of age)	11.98(0.27)	11.91(0.37)	11.98(0.35)	11	13	11.96(0.33)
Gender:						
Females	24	18	16	-	-	58
Males	19	17	28	-	-	64

Baseline Characteristic	Control (SD) (n=42)	HF Mean (SD) (n =47)	LF Mean (SD) (n =47)	Total Min (n =136)	Total Max (n =122)	Total Mean (SD) (n =122)
Anthropometrics:						
Weight (kg)	44.97(14.09)	47.581(10.67)	47.44(11.79)	26	85	46.66(12.33)
Height (cm)	151.21(9.52)	154.41(8.02)	154.79(8.15)	128	170	153.39(8.72)
% Family income (TB/M)						
0-5,000	9.30	11.43	11.36	-	-	10.66
5,001-10,000	41.86	22.86	38.64	-	-	32.25
10,001-15,000	18.60	11.43	11.36	-	-	13.93
15,001-20,000	13.95	14.29	22.73	-	-	17.21

Baseline Characteristic	Control (SD) (n=42)	HF Mean (SD) (n =47)	LF Mean (SD) (n =47)	Total Min (n =136)	Total Max (n =122)	Total Mean (SD) (n =122)
20,001-25,000	11.63	8.57	2.73	-	-	7.38
25,001-30,000	2.33	8.57	2.73	-	-	4.10
More than 30,000	2.33	20.00	6.81	-	-	9.02
	0	2.86	4.55			2.46
Number of family members	5.26(1.95)	4.91(1.50)	4.70(2.13)	2	13	4.97(1.90)
Grade Point Average From Grade 5	3.10(0.64)	3.17(0.65)	3.00(0.76)	1.47	4.00	3.09(0.69)

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; TB/M= Thai Baht per month.

Table 4-2 Diary Food Record of participants.

	Control (SD) (n=42)	HF Mean (SD) (n =47)	LF Mean (SD) (n =47)	Total Min (n =136)	Total Max (n =136)	Total Mean (SD) (n =136)
Total flavonoids from intervention fruits µg/day	0.00	13,257.70(2889.77)	1,032.83(271.27)	180.28	18,703.37	7,215.53(6,480.94)

4.4.2 Daily Food record and Food Frequency Questionnaire

The total amount of flavonoids from intervention fruits were estimated as 7,215.53 µg per day (SD=6,480.94), with HF and LF averaging 13,257.70 µg per day (SD=2,889.77) and 1,032.82 µg per day (SD=271.27) respectively show in Table 4-2.

Data from the Food Frequency Questionnaire revealed that the average numbers of the food portions consumed for each food group and are shown in Table 4-3. The One-Way ANOVA revealed that there was only the statistic difference in FFQ 3 in candy consumption with higher intake in HF participants than control participants and LF participants (HF=0.84; Control=0.54; LF=0.40) (F (2,119) = 3.456, p =.035) and no any other significant difference between group in food intake.

Food Frequency Questionnaire revealed that the average numbers of portions of fruit and vegetables in the 1st, 2nd, and 3rd FFQ were 6.25 (SD=3.75), 6.33 (SD=3.75) and 6.40(SD=3.78) in HF, 6.72 (SD=3.49), 7.54 (SD=4.95) and 7.52 (SD=6.45) in LF and 8.36 (SD=3.75), 7.98 (SD=4.15) and 7.79 (SD=4.04) in control group.

As can be seen in Table 4-4, fruit and vegetable consumption (including the intervention fruit) was not significantly different between 3 conditions (F (1,159) =2.228, p =.106). This was similarly the case when the groups were analysed separately for fruit (F (1,159) =1.512, p =.225) and vegetable intake (F (1,159) =1.673, p =.192). No significant differences were observed even when excluding the control group from the analysis with fruit and vegetable intake (F (1,159) =1.330, p =.252), fruit consumption (F (1,159) =1.084, p =.301), and vegetable consumption (F (1,159) =0.719, p =.399) (see in Table 4-5).

Table 4-3 Portion of food consumption from Food Frequency Questionnaire of participants

	Control Mean(SD)	HF Mean(SD)	LF Mean(SD)	*F*	*p*
Food Frequency Questionnaire:					
Carbohydrate Food Group;					
1stFFQ	6.88(4.36)	6.55(4.20)	7.95(4.42)	1.236	.294
2ndFFQ	6.43(3.58)	5.96(3.94)	7.21(4.63)	1.017	.365
3rdFFQ	6.05(4.65)	5.08(3.70)	5.51(3.99)	0.542	.583
Meat and Protein Food Group					
Meat					
1stFFQ	7.02(4.67)	7.93(6.50)	7.14(5.15)	0.330	.720
2ndFFQ	6.98(5.06)	7.63(5.50)	6.33(4.28)	0.742	.479
3rdFFQ	5.83(4.92)	5.95(4.10)	6.70(5.01)	0.423	.656
Milk					
1stFFQ	1.85(1.01)	1.94(1.40)	2.17(2.17)	0.413	.662
2ndFFQ	2.16(1.57)	1.81(1.48)	1.90(1.50)	0.541	.584
3rdFFQ	1.80(1.31)	1.96(1.28)	2.39(2.85)	0.930	.396

	Control Mean(SD)	HF Mean(SD)	LF Mean(SD)	*F*	*p*
Soy					
1stFFQ	1.55(1.49)	1.27(1.45)	1.74(2.56)	0.653	.522
2ndFFQ	1.39(1.74)	1.16(1.13)	0.91(0.91)	1.404	.250
3rdFFQ	1.39(2.18)	1.05(0.91)	1.11(0.84)	0.661	.518
Egg and Bean					
1stFFQ	3.25(2.72)	2.81(2.11)	2.86(2.13)	0.394	.675
2ndFFQ	2.61(1.44)	2.17(1.36)	2.41(1.90)	0.747	.476
3rdFFQ	2.21(1.79)	2.11(1.63)	2.14(1.52)	0.042	.959
Fruits and Vegetable Food Group					
Vegetable					
1stFFQ	3.94(2.33)	2.83(2.20)	3.38(2.48)	2.187	.117
2ndFFQ	3.60(2.76)	3.03(2.42)	2.95(1.94)	0.842	.434
3rdFFQ	3.28(2.42)	2.67(1.61)	3.17(3.01)	0.757	.471
Fruit					
1stFFQ	4.42(2.33)	3.69(2.57)	3.34(1.75)	2.303	.104
2ndFFQ	4.38(2.47)	3.30(2.22)	4.60(4.16)	2.139	.122
3rdFFQ	4.51(2.90)	3.73(3.02)	4.35(4.41)	0.551	.578

	Control Mean(SD)	HF Mean(SD)	LF Mean(SD)	*F*	*p*
Juice and snack Food					
Group					
Juice					
1stFFQ	1.04(0.75)	1.38(1.74)	0.94(1.34)	1.191	.307
2ndFFQ	0.90(0.84)	1.11(1.62)	1.00(1.31)	0.266	.767
3rdFFQ	0.75(0.76)	0.87(0.77)	0.99(0.97)	0.814	.446
Snack and Dessert					
1stFFQ	2.47(1.63)	2.40(2.33)	3.23(2.84)	1.589	.208
2ndFFQ	2.59(1.41)	2.45(2.12)	2.61(1.92	0.093	.912
3rdFFQ	2.83(2.17)	2.01(1.25)	2.23(1.97)	2.091	.128
Soft Drink					
1stFFQ	1.57(1.71)	1.83(1.82)	1.42(1.90)	0.591	.556
2ndFFQ	1.38(1.31)	1.43(1.50)	0.82(1.09)	2.833	.063
3rdFFQ	1.31(1.40)	1.22(1.07)	1.09(1.13)	0.326	.723
Candy					
1stFFQ	1.36(1.87)	1.36(1.63)	1.26(2.15)	0.041	.960
2ndFFQ	0.57(0.64)	0.65(0.82)	0.60(1.12)	0.083	.920
3rdFFQ	0.54(0.66)	0.84(1.14)	0.40(0.41)	3.456	.035*

Table 4-4 Mean and standard deviation of fruit, vegetable and fruit and vegetable consumption in portion from semi-quantitative food frequency questionnaire for all groups

Group	FFQ 1 Mean(portion) Sd	FFQ 2 Mean(portion) Sd	FFQ 3 Mean(portion) Sd	*F*	*p*
FV					
1(C)	8.36(3.75)	7.98(4.15)	7.79(4.04)	T:0.020	.980
2(HF)	6.52(3.75)	6.33(3.75)	6.40(3.87)	G:2.288	.106
3(LF)	6.72(3.49)	7.54(4.95)	7.52(6.45)	TXG:0.543	.704
V					
1(C)	3.94(2.33)	3.60(2.76)	3.28(2.42)	T:1.064	.347
2(HF)	2.83(2.20)	3.03(2.42)	2.67(1.61)	G:1.673	.192
3(LF)	3.38(2.48)	2.95(1.94)	3.17(3.01)	TXG:0.607	.658
F					
1(C)	4.42(2.33)	4.38(2.47)	4.51(2.90)	T:0.775	.462
2(HF)	3.69(2.57)	3.30(2.22)	3.73(3.02)	G:1.512	.225
3(LF)	3.34(1.75)	4.60(4.16)	4.35(4.41)	TXG:1.346	.254

Table 4-5 Mean and standard deviation of fruit, vegetable and fruit and vegetable consumption in portion from semi-quantitative food frequency questionnaire for HF and LF groups

Group	FFQ 1 Mean(portion) Sd	FFQ 2 Mean(portion) Sd	FFQ 3 Mean(portion) Sd	*F*	*p*
FV					
1(HF)	6.52(3.75)	6.33(3.75)	6.40(3.87)	T:0.249	.780
2(LF)	6.72(3.49)	7.54(4.95)	7.52(6.45)	G:1.330	.252
				TXG:0.533	.588
V					
1(HF)	2.83(2.20)	3.03(2.42)	2.67(1.61)	T:0.238	.789
2(LF)	3.38(2.48)	2.95(1.94)	3.17(3.01)	G:0.719	.399
				TXG:0.824	.440
F					
1(HF)	3.69(2.57)	3.30(2.22)	3.73(3.02)	T:1.012	.365
2(LF)	3.34(1.75)	4.60(4.16)	4.35(4.41)	G:1.084	.301
				TXG:2.149	.120

4.4.3 Analysis of all groups

4.4.3.1 O-NET score

The O-NET performance in all groups were similar (control=0.415; HF= 0.420; LF= 0.418). To compare the O-Net scores performance at the end of intervention, a one-way-measure of ANOVA was used. As can be seen in Figure 4-3 no significant differences were found between the three groups (F (2,119) =0.020, p=.980).

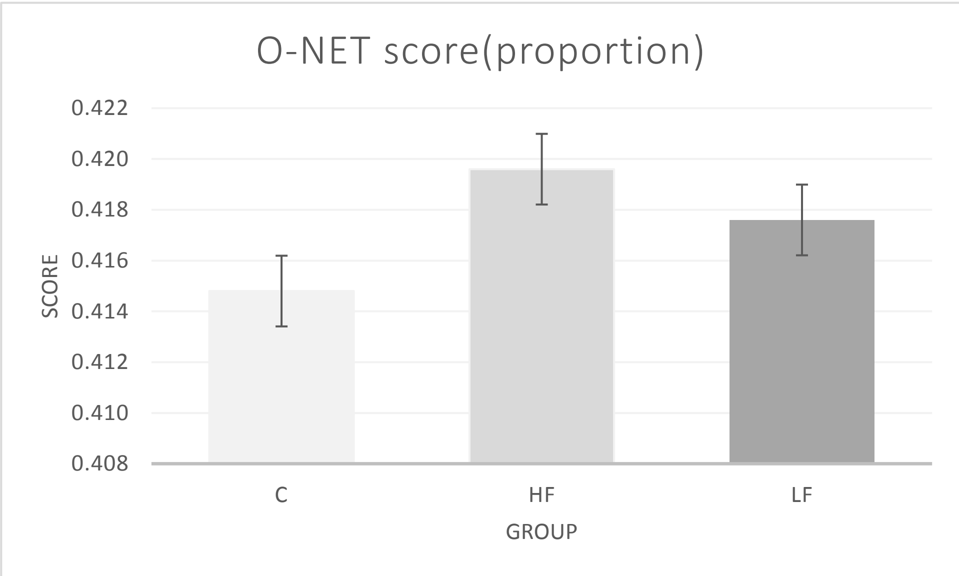

Figure 4-3 Mean and standard error for proportion of total 8 subject of O-Net score in all groups of participants.

4.4.3.2 Composite score of executive function

To assess the composite score for executive function to be compared directly to the previous experiment, a z score of just the flanker task interference effect and letter memory response time were combined. As can be seen in Table 4-6 all groups performed better over the course of the intervention time with the control group comprising of z scores of baseline and post intervention (control = 0.13 and -0.12; HF =0.16 and -0.17; LF= 0.06 and -0.06). However, there was only a statistic difference in the main effect of time (F (2,119) =12.389, p<.001), with no difference in the main effect of group (F (2,119) =0.004, p=.996), or critically, the time x group interaction (F (2,119) =0.923, p=.400).

In this experiment, the StopGo task was also employed to measure executive function. A further composite score of EF was created which combined the z score of flanker task interference effect, letter memory response time and StopGo response time. The mean score of EF in control and HF groups were better over time (control = 0.07 and -0.09; HF= 0.09 and -0.13 in HF) but there was slightly change in the LF (0.02 and 0.03). A repeated measure ANOVA revealed that only a main effect of time was revealed to be statistically significant (F (2,119) =3.936, p=.050), but no main effect of group (F (2,119) =0.070, p=.932), or a time x group interaction (F (2,119) =1.307, p=.274).

The results obtained from executive function analysis are shown in Figure 4-4 and Figure 4-5.

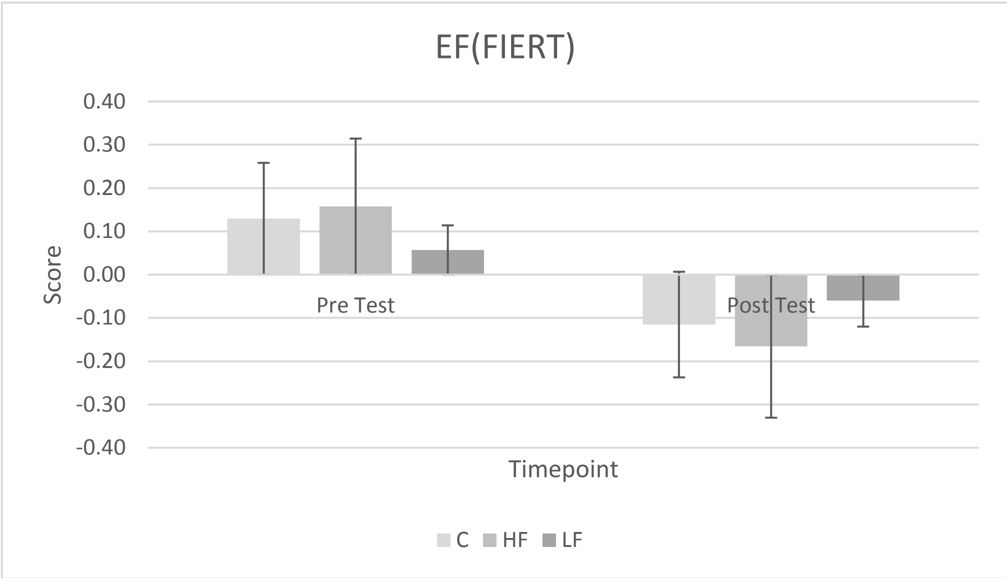

Figure 4-4 Composite score of Executive function (±standard error of the mean) (z score of flanker task interference effect, letter memory response time)

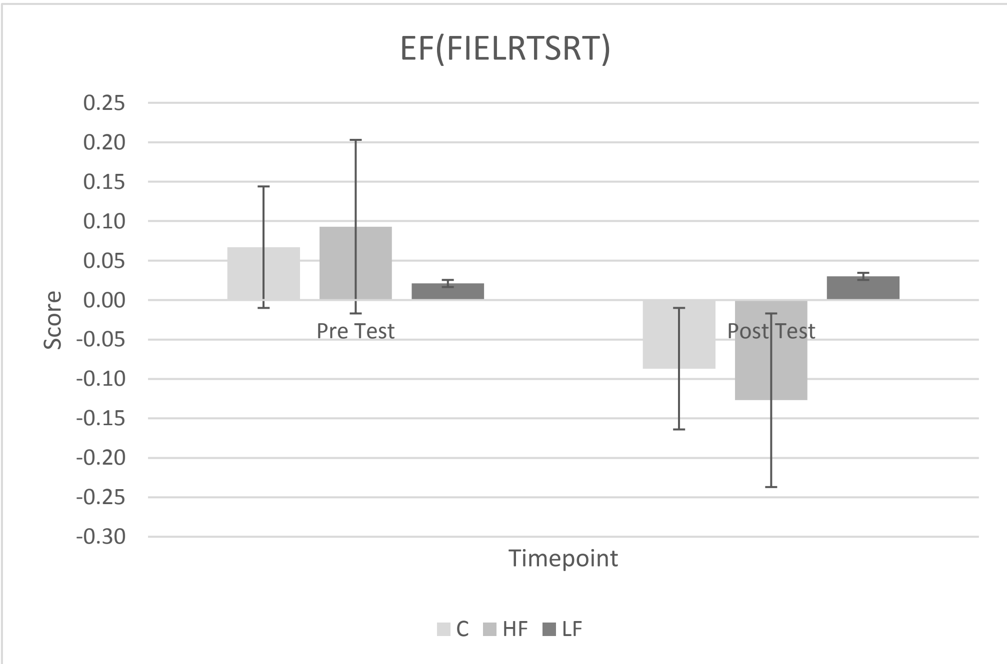

Figure 4-5 Composite score of Executive function (±standard error of the mean) (z score of flanker task interference effect, letter memory response time and StopGo response time)

4.4.3.3 PANAS Mood

In comparing the positive affect score between baseline and post-intervention, all groups were got worse (less positive) but displayed less of a drop in the HF group (control=31.43 and 30.69; HF= 33.59 and 33.57; LF= 33.63 and 31.58). In order to measure the effect of intervention, repeated-measures ANOVAs were used. There were no significant differences in a main effect of time (F (2,119) =2.747, p=.100), group (F (2,119) =1.884, p=.156), or time x group interaction (F (2,119) =1.184, p=.310).

The average score for negative affect, at baseline was higher for the high flavonoid participants than control and low flavonoids participants (HF=23.38 versus control=21.90 and LF=21.79) but at the end of the intervention high flavonoids participants showed a greater reduction than low flavonoids participants (HF=22.14 versus LF=21.74) with increasing negative affect in the control group (22.52). This trend repeated the result of Experiment 1 in that the HF group showed more decrease in negative affect compared with LF. Disappointingly, though, as can be seen in Table 4-6., there was no statistical significance in the main effect of time (F (2,119) =0.141, p=.708), group (F (2,119) =0.333, p=.718), or time x group interaction (F (2,119) =0.842, p=.433).

Table 4-6 Composite score of cognitive and Mood Measures: Z score, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all groups.

Cognitive/Mood Variable	Time	Control MeanZ(SD)	HF MeanZ(SD)	LF MeanZ(SD)	Repeated Measures ANOVA *F*	*p*
PANAS	Baseline	31.43(6.25)	33.59(6.35)	33.63(6.29)	T:2.747	.100
Positive affect	Post-Intervention	30.69(6.80)	33.57(5.96)	31.58(7.46)	G:1.884	.156
					TXG:1.184	.310
Negative affect	Baseline	21.90(5.88)	23.38(6.05)	21.79(7.05)	T:0.141	.708
	Post-Intervention	22.52(7.13)	22.14(7.14)	21.74(5.91)	G:0.333	.718
					TXG:0.842	.433

Cognitive/Mood Variable	Time	Control MeanZ(SD)	HF MeanZ(SD)	LF MeanZ(SD)	Repeated Measures ANOVA	
					F	*p*
O-NET Score		0.415(0.10)	0.420(0.10)	0.418(0.12)	0.020	.980

4.4.3.4 Individual result of Individual cognitive task/mood from all groups

4.4.3.4.1 Letter Memory

In looking at letter memory response times both at baseline and post-intervention, LF participants performed better than control group and HF (baseline LF=2579, control= 2843 and HF=2974; post-intervention LF=2230, control=2305 and HF=2301). There was a statistically significant main effect of time (F (2,119) =32.878, $p<.001$) but no main effect of group (F (2,119) =1.336, p=.267), or a time x group interaction (F (2,119) =1.138, p=.324).

4.4.3.4.2 Flanker Task

For the flanker interference effect, high flavonoids participants were better both in baseline and post intervention (23.24 and 26.98) than control (26.39 and 30.61) and low flavonoids participants (31.67 and 37.15). However, although this is consistent with the trend observed in Experiment 1, there was no significant effect for the main effects of time (F (2,119) =1.631, p=.204), group (F (2,119) =1.167, p=.315), or time x group interaction (F (2,119) =0.023, p=.977).

4.4.3.4.3 StopGo Task

In term of StopGo task all groups performed very similarly in response time at baseline (control = 533, HF = 535 and LF=533) but at the end of intervention low flavonoids group (556) were worse than the high flavonoid (533) or control group (535). Again, similar to the other executive function tasks in this experiment there was no statistically significant difference in the main effects of time (F (2,119) =0.749, p=.388), group (F (2,119) =0.307, p=.736), or time x group interaction (F (2,119) =0.715, p=.491).

As can be seen in Table 4-7 below, the mean individual score of Letter memory and Flanker tasks and StopGo.

Table 4-7 Individual score of cognitive task: Mean, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all conditions.

Cognitive/Mood Variable	Time	Control Mean(SD)	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
Letter Memory Task	Baseline	2843(913.64)	2974(799.61)	2579(876.25)	T:32.878	.000*
Letter RT	Post-Intervention	2301(914.84)	2305(723.86)	2230(876.97)	G:1.336	.267
					TXG:1.138	.324
Flanker Task	Baseline	26.39(32.66)	23.24(36.99)	31.67(29.75)	T:1.631	.204
Flanker Interference Effect	Post-Intervention	30.61(28.54)	26.98(39.42)	37.15(36.95)	G:1.167	.315
					TXG:0.023	.977

Cognitive/Mood Variable	Time	Control Mean(SD)	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA	
					F	*p*
StopGo						
RT	Baseline	533(88.84)	535(86.45)	533(83.06)	T:0.749	.388
	Post-Intervention	535(88.67)	533(80.11)	556(89.77)	G:0.307	.736
					TXG:0.715	.491

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; T= Time; G= Flavonoids treatment group; TxG= time x Flavonoids treatment group interaction. *p<.05.

4.4.4 Analysis of HF versus LF

To enable a direct comparison with Experiment 1, analysis of HF and LF groups without the control group was employed. The result of two group's analysis was presented below.

4.4.4.1 O-NET score

The mean proportion score of the O-NET in both groups were similar (0.418 versus 0.420 in HF and LF group respectively). To compare the O-Net score (proportion score of 8 subjects) at the end of intervention, an independent t-test measure was used. No significant differences were again found between two groups (*t* (85) =0.090, *p*=.929).

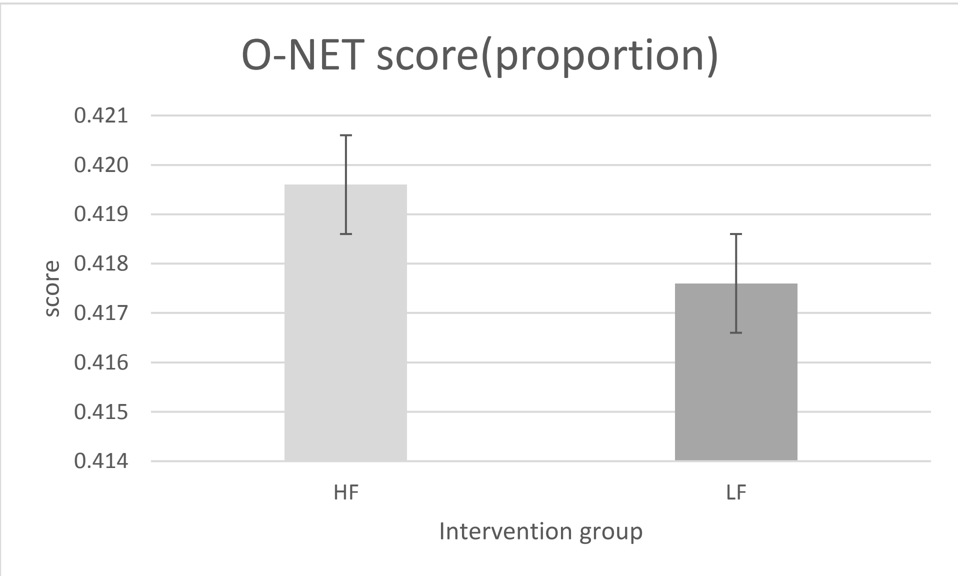

Figure 4-6 Mean and standard error for proportion of total 8 subject of O-Net score in all groups of participants

4.4.4.2 Composite score of executive function

Analysing the composite score for the flanker interference effect and letter memory response time showed that both groups performed better over time with HF 0.16 and -0.17, and LF 0.06 and -0.06. However, there were no statistically significant differences except for a main effect of time (F (1, 85) = 8.123, p=.005), with no difference for the main effect of group (F (1, 85) =0.000, p=.985), or a time x group interaction (F (1, 85) =1.781, p=.186).

A further executive function analysis was conducted which comprised a combined z score of flanker task interference effect, letter memory response time and StopGo response time. The mean score of EF in HF groups was worse than LF at baseline (0.09 versus 0.02) but better over time (-0.13 versus 0.03). A repeated ANOVA revealed that no statistically significant differences in the main effect of time (F (1, 85) =2.525, p=.116), a main effect of group (F (1, 85) =0.126, p=.723), or time x group interaction (F (1, 85) =2.945, p=.090).

The results obtained from executive function analysis in HF and LF groups are shown in Table 4-8.

4.4.4.3 PANAS Mood

In comparing the positive affect scores between baseline and post-intervention, both group got worse but displayed less of a drop in the HF group (HF=33.59 versus 33.57, and LF=33.63 versus 31.58). In order to measure the effect of intervention, repeated-measures ANOVAs were used. There were no significant differences for time (F (1, 85) =2.479, p=.119), group (F (1, 85) =0.620, p=.433), or time x group interaction (F (1, 85) =2.380, p=.127).

The average score of negative affect at baseline showed that high flavonoid participants displayed higher scores than low flavonoids participants (23.38 versus 21.79) but at the end of the intervention high flavonoids participants displayed a greater reduction (indicating less negative mood) than low flavonoids participants (22.14 versus 21.74). This trend repeated the result of Experiment 1 in that the HF group more decrease in negative affect to compare with LF. As can be seen in Table 4-8, there was no statistic significant in the main effect of time (F (1, 85) =0.875, p=.352), group (F (1, 85) =0.654, p=.421), or time x group interaction (F (1, 85) =0.753, p=.388).

Table 4-8 Composite score of cognitive and Mood Measures: Z score, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for only HF and LF participants.

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	 *p*
Executive Function:	Baseline	0.16(0.69)	0.06(0.72)	T:8.123	.005*
(FIE&LRT)	Post-Intervention	-0.17(0.73)	-0.06(0.61)	G:0.000	.985
				TXG:1.781	.186
Executive Function:		0.09(0.64)	0.02(0.71)	T:2.525	.116
(FIE&LRT&SRT)		-0.13(0.63)	0.03(0.57)	G:0.126	.723
				TXG:2.945	.090

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
PANAS	Baseline	33.59(6.35)	33.63(6.29)	T:2.479	.119
Positive affect	Post-Intervention	33.57(5.96)	31.58(7.46)	G:0.620	.433
				TxG:2.380	.127
Negative affect	Baseline	23.38(6.05)	21.79(7.05)	T:0.875	.352
	Post-Intervention	22.14(7.14)	21.74(5.91)	G:0.654	.421
				TxG:0.753	.388
O-NET Score		0.420(0.10)	0.418(0.12)	T(85)=0.090	.929

4.4.4.4 Individual result of Individual cognitive task from all groups

4.4.4.4.1 Letter Memory

In terms of letter memory response time, HF participants performed better over the course of intervention time (2974 at baseline versus 2305 post-intervention) than LF participants (2579 versus 2230). There was a statistically significant in the main effect of time (F (1, 85) =21.133, p<.001) but no main effect of group (F (1, 85) =2.950, p=.089), or a main effect of time x group interaction (F (1, 85) =2.097, p=.151).

4.4.4.4.2 Flanker Task

In terms of the flanker interference effect (subtracting response time of incongruent trials ability from congruent trials ability) high flavonoids participants were better both in baseline (HF=23.24 ms versus LF=31.67) and post intervention (HF=26.98 ms versus LF=37.17) than low flavonoids participants. However, there was no significant effects of time (F (1, 85) =1.293, p=.295), group (F (1, 85) =2.008, p=.160), or a time X group interaction (F (1, 85) =0.046, p=.831).

4.4.4.4.3 StopGo Task

In terms of StopGo performance both groups performed very similarly in response time at baseline (HF = 535 ms versus LF=533 ms) but at the end of intervention low flavonoids group got worse than high flavonoids (LF=556 ms versus HF=533 ms). Again, no statistically significant differences of time (F (1, 85) =1.143, p=.288), group (F (1, 85) =0.469, p=.495), or a time X group interaction (F (1, 85) =1.458, p=.231) were observed.

As can be seen in Table 4-9 below, the mean individual score of Letter memory and Flanker task and StopGo.

Table 4-9 Individual score of cognitive task: Mean, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for only HF and LF conditions.

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
Executive Function:					
Letter Memory Task	Baseline	2974(799.61)	2579(876.25)	T:21.133	.000*
Letter RT	Post-Intervention	2305(723.86)	2230(876.97)	G:2.950	.089
				TxG: 2.097	.151
Flanker Task	Baseline	23.24(36.99)	31.67(29.75)	T:1.293	.259
Flanker Interference Effect	Post-Intervention	26.98(39.42)	37.15(36.95)	G:2.008	.160
				TxG:0.046	.831

Cognitive/Mood Variable	Time	HF Mean(SD)	LF Mean(SD)	Repeated Measures ANOVA *F*	*p*
StopGo					
RT	Baseline	535(86.45)	533(83.06)	T:1.143	.288
	Post-Intervention	533(80.11)	556(89.77)	G:0.469	.495
				TxG:1.458	.231

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; T= Time; G= Flavonoids treatment group; TxG= time x Flavonoids treatment group interaction. *$p<.05$.

4.5 Discussion

The main aim of the study was to investigate whether academic attainment, cognitive performance (focused on executive function) and mood could be affected by a flavonoid rich, fruit based intervention. Double portions of intervention fruits in this study were expected to clarify the relationship of flavonoids on cognitive performance in primary school age group that was initially shown in Experiment 1. That previous study had shown positive effect of flavonoid rich fruits on a composite score of executive function, and a negative effect on negative affect but no association on the main O-Net score or positive affect. The results of the current study have shown a similar trend in terms of the direction of performance, but overall have not replicated Experiment 1. As in Experiment 1, there was no significant improvement in HF participants in terms of O-Net performance. In contrast though to Experiment 1, the executive function and mood measures failed to reveal any significant difference between groups.

The main executive function analysis failed to reveal any statistically significant differences as a function of flavonoid regime across the three groups, with a similar picture emerging from a high versus low flavonoid analysis (excluded control condition) which was conducted to allow direct comparison to Experiment 1. Thus, there were no reliable flavonoid related benefits across any of these measures employed here.

A number of epidemiology studies of plant based flavonoids in diets have shown positive effects on different cognitive domains (Butchart et al., 2011; Kang et al., 2005; Letenneur et al., 2007; Morris et al., 2006; Nooyens et al., 2011; Nurk et al., 2009, 2010; Pastor-Valero et al., 2014; Peneau et al., 2011; Polidori et al., 2009; Ye et al., 2013; Yuan et al., 2014). However, there have been limit studies employing RCTS with which to compare our results.

Only Kean et al. (2015) previously carried out an intervention of high flavonoid orange juice, showing an effect on global cognitive function (mean of all test combined). Therefore, the current study might be best viewed as an exploratory pilot to investigate the benefit of long term supplementation of fruits based flavonoids to promote cognitive performance in school age groups, particularly to improve academic outcome.

There are several possible explanations for the absence of any significant results. The response inhibition task comprising the Stop-Go employed in this study may be less sensitive to flavonoid intervention in children. The previous intervention of anthocyanin supplementation with 7-10 year old children was carried out by Whyte et al. (2015) revealed no significant effect for any of the response inhibition tasks. A further possible explanation might be that even though we offered low and high flavonoids fruits to children in two of the groups, the portion of fruits and vegetable consumption calculated from the FFQ during the intervention showed no difference between three groups (i.e., compared to those children that were offered no additional fruits). This suggests that the high and low flavonoid group children might be been compensating for eating additional portions of fruit at school by not eating further portions (that they would normally eat) at home. Whilst the current study was not set up to explore this issue directly the net effect would be that the control participants likely had similar micro or macro nutrients from fruit and vegetables to the fruit based children which may explain the lack of significant cognitive differences.

In terms of mood effects, analysis of all participants revealed that the high flavonoid group maintained a positive affect score for the duration of the trial, while low flavonoid and control group participants displayed a drop off by the end of intervention. The beneficial of flavonoids may show in negative affect as well in that only in the HF condition was negative affect reduced at the end of intervention whereas LF condition remained and indeed increased in the case of the control condition. However, the current result failed to show significant effects of flavonoid rich fruits and mood effects as found in Experiment 1. This is consistent with Kean et al. (2015) who revealed no significant difference in positive or negative affect from supplementation of high flavanones 100 % orange juice containing 305 mg flavanone during 8 weeks intervention in older adult.

In terms of the analysis of O-Net score as the real-life measure of current study, we again failed to find a differential effect of flavonoid supplementation on this measure. One study that did show an effect of nutrition supplementation on academic performance was conducted by Eilander et al. (2010),. They reviewed the literature of multiple micronutrient on cognitive performance in school age children, which demonstrated that a marginal increase in fluid intelligence and academic performance may be related to multiple micronutrient supplementation. Thus, to improve academic outcome in schoolchildren one could speculate that it by necessary focus on multiple micronutrient supplementation rather than on a single micronutrient such as flavonoids.

Finally, one should also acknowledge that the real world testing environment of the school may have had an impact on our ability to detect relatively small changes in cognitive performance in the present study (e.g., noise). Coupled with the relatively small numbers of participants per condition (power =0.81), it is possible that this contributed to our failure to replicate the effects previously demonstrated in Experiment 1 (power =0.95).

In summary, therefore, the results for Chapter 4 together with those in Chapter 3, provide mixed evidence that flavonoids supplementation had positive association on cognitive performance. In the next chapter, we report an experiment which employed a more detailed executive function test battery and a greatly increased number of participants.

Chapter 5 Effect of 12 weeks supplementation of flavonoid-rich fruits on executive function and mood in children aged 11-13 years old

5.1 Introduction

Chapter 3 reported an effect of flavonoid-rich fruit supplementation on cognitive performance particularly executive function (EF), and a reduction in negative affect. In contrast, whilst demonstrating a similar pattern of effects, Chapter 4 failed to detect significant differences as a function of flavonoid status across an expanded range of executive function tests. One major difference across experiments, was that a relatively smaller number of participants were assigned to each condition in Chapter 4 (35-40 participants per groups) which may account for the discrepant findings. In the current chapter, to achieve greater power to detect, what are after all small effects, larger groups of children were allocated to each group to examine flavonoids-related effects on O-Net test, an EF battery and mood measures. In terms of EF, previously in Chapter 3 and 4, computer based versions of a Flanker task, letter memory task and Stop-Go task were used to investigate the flavonoids effect. However, in scaling up the numbers of participants per group one practical constraint is access to sufficient numbers of computers to allow the testing of children within the context of the typical school day.

Therefore, for the current study, the decision was taken to employ paper and pencil versions of the Digit Symbol Substitution Test (DSST), Trail Making Test (TMT), Colour-Word Interference Test (Stroop), and Verbal Fluency Test (VF) to measure EF in grade 6 student aged 11-13 in Saun-Sanook municipal school in Khon-Kaen province, Thailand.

The DSST is used to measure response processing speed, attention, visual spatial skill and shifting skill (Pradhan & Nagendra, 2009; Van der Elst et al., 2012). The D-KEFS (Delis-Kaplan Executive Function System) is a set of executive function tests that includes the Trail Making Test, Verbal Fluency Test, Design Fluency Test, Color-Word Interference Test, Sorting Test, Twenty Question Test, Word Context Test, Tower Test, and Proverb Test. The D-KEFS is a game-like test that is designed to be free from right or wrong answers that serves to reduce unproductive discouragement and frustration of participants from receiving negative feedback. The test suits ages from 8-89 years old and can be use individually or in group settings (Homack, Lee, & Riccio, 2005). In the current study, we selected to use a subset of those tests given time constraints, namely the TMT, Stroop and VF.

In a previously published study, the trail Making Test (TMT) A and B, and the Verbal Fluency Test (VFT) were employed to measure cognitive performance at baseline and the end of an 8 week intervention (Mastroiacovo et al., 2015). The results showed that high flavonoid (HF) and intermediate flavonoid (IF) groups in comparison to low flavonoid (LF) were significantly quicker to complete the TMT A and B. The pattern was also observed for VFT scores such that the HF group perform better than IF and LF. Kean et al. (2015) also conducted an 8 week crossover, double-blind randomized in healthy older adult (average age 67) which supplemented high flavanones 100 % orange juice containing 305 mg flavanones. Cognitive performance was measured by the Go-NoGo, DSST and the letter memory to evaluate executive function and the CERAD to evaluate episodic memory. Only global cognitive function (mean of all test combined) showed sensitivity to the effects of the flavanone rich orange juice.

In further flavonoids interventions Crews Jr et al. (2008b) conducted a 6 week supplementation of either flavonoids- and procyanidine dark chocolate (proanthocyanidins 397.30 mg from chocolate bar and 357.41 mg from cocoa drink) or artificially sweetened matched placebo (proanthocyanidins 0.20 mg from chocolate bar and 40.87 mg from cocoa drink) in 60 year old participants. In contrast to results from other studies, they reported no significant differences between the two groups in cognitive tasks including a selective reminding test, Wechsler Adult Intelligence Scale-III, Wechsler Memory Scale-III, TMT and Stroop Colour-Word Test. In summary, existing findings indicate some cognitive test sensitivity to chronic flavonoid supplementation across tasks and flavonoid groups.

In this chapter, participants were allocated into two group high flavonoids (HF) and low flavonoids (LF) in an attempt to replicate the findings from Chapter 3. However, the portion of fruit offered was the same as in Chapter 4 (80 grams portion). The overall aim was to examine whether HF versus LF intake significantly affected EF, O-Net test and mood in grade 6 student in Saun-Sanook municipal school Khonkaen, Thailand. In term of other measures, the PANAS mood scale remained to evaluate positive and negative affect in relation to flavonoids supplementation. The O-Net score was still the main measure of real-life academic attainment as in previous chapters.

5.2 Methods

5.2.1 Participants

Participants were 404 primary school grade 6 students (age 10-13 years old) from Saun-sanook Municipal school studying in the 2015 academic year, from Khonkaen province in Thailand. There were 3 programmes in the school consisting of 5 classes of ordinary programmes, 3 classes of English programme and 2 classes of gifted programmes. Each class contained 44-46 students. For this experiment, children were randomly allocated to a low flavonoid (LF) group or high flavonoid (HF) group (n=~20-22 per group for each class). In total, 202 students were allocated to the HF group and 202 students to the LF group. Over the course of the experiment, participants who consumed less than 50 percent of the intervention fruit were excluded from the final analysis. Furthermore, participants that completed less than 75 percent of the cognitive tasks were also removed from the analysis. Therefore, there were 175 participants in HF group and 183 participants in LF group in the final analysis. Table 5-1 shows the demographics and key characteristics of the remaining participants.

5.2.2 Intervention Fruits

In Experiment 1 (Chapter 3) participants additionally received a flavonoid-rich or flavonoid-poor fruit (approximately 40-50 grams) after their main school meal, and in the Experiment 2 (Chapter 4), low flavonoids and high flavonoids were supplemented with an additional 80 grams of intervention fruit, while the control group was offered 1 portion of biscuit or bun (70-80 calories). In this experiment (Experiment 3) participants were offered 80 grams of intervention fruits in the flavonoid-rich group consisting of pomelo, guava, pineapple and orange over the course of the intervention, whereas the flavonoid-poor group consumed fruits such as watermelon, melon, papaya and banana (USDA, 2007). The fruit interventions were administered daily by the class teachers, and all children recorded their daily intake during the intervention. The content of flavonoids in the respective fruits is shown in Chapter 3.

5.2.3 Cognitive Tests

5.2.3.1 Digit Symbol Substitution Test

The DSST measures response processing speed, attention, visuo-spatial skills and shifting skills and has been employed previously for use in school aged children (Pradhan & Nagendra, 2009; Van der Elst et al., 2012).The test requires the participant to complete as many unique geometric symbols with their corresponding Arabic numbers within a time limit of 90 seconds. As can be seen in Figure 3.1, the digit symbol codes were given at the top of the paper and 118 empty squares which were randomly numbered 1-9 for participants to complete with the corresponding symbol. In this version of the task, participants were asked to complete as many of the boxes as they could with the symbol corresponding to the number given. Participants were requested to start in the first row, working in sequence from left to right, without missing out any of the squares. Participants were given 90 seconds for this task. Therefore, participants were scored for each of the item correctly completed. Two alternate versions of this task were employed and randomly assigned for test either pre-test or post-test.

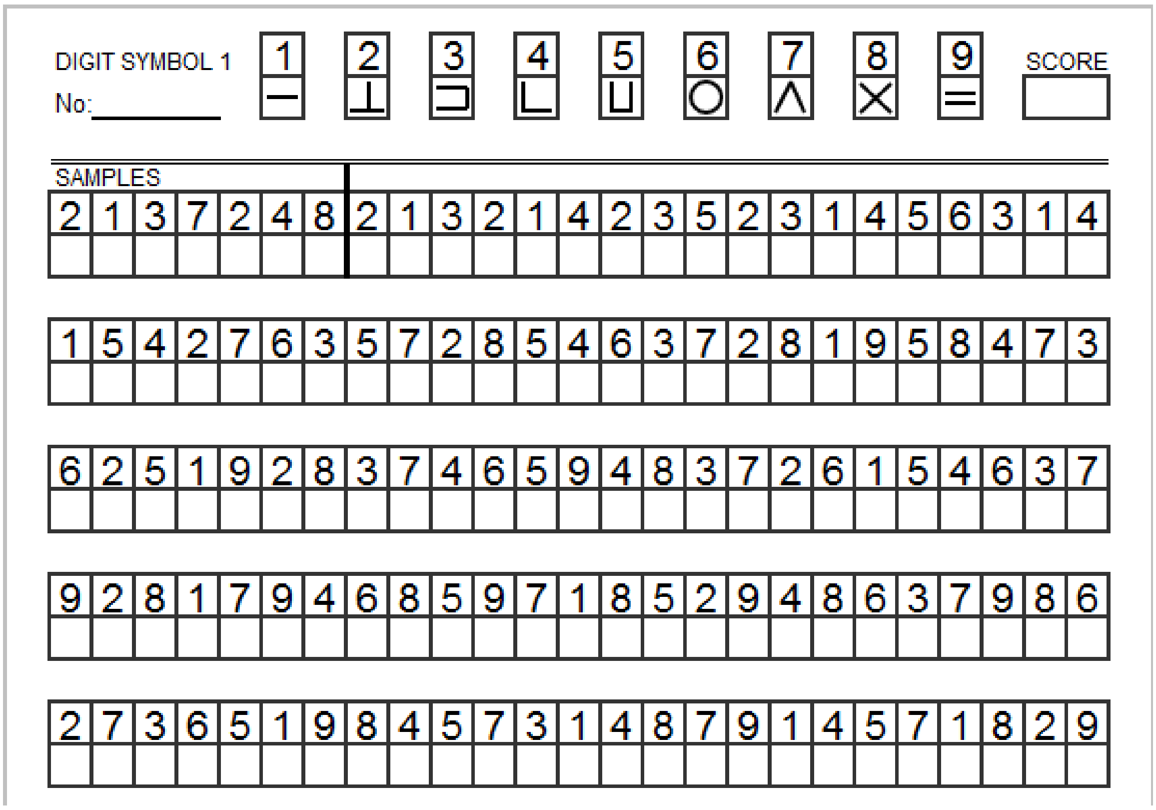

Figure 5-1 Example of DSST.

5.2.3.2 Trail Making Test

The Trail Making Task is one component of the D-KEFS (Delis-Kaplan Executive Function System), which is a set of executive function tests that included the trail Making Test, Verbal Fluency Test, Design Fluency Test, Color-Word Interference Test, Sorting Test, Twenty Question Test, Word Context Test, Tower Test, and the Proverb Test. The D-KEFS is a game-like test free from right or wrong answers that can reduce unproductive discouragement and frustration of participants that can occur when given negative feedback. The test suit 8-89 year old sand can be use individually or as part of a group (Homack et al., 2005).

The TMT task is used to measure flexibility of thinking and consists of visual search speed, scanning, and speed of processing and mental flexibility components. The original version of the test commonly consists of two parts: TMT A requires participants to connect a set of 25 dots of numbers (1,2,3…) in sequential order as fast as they can and for TMT B they are instructed to connect both numbers and letter (1,A,2,B,3,C…) in sequential order(Chaytor, Schmitter-Edgecombe, & Burr, 2006). In this study, 3 sets of TMT A and Thai letters TMT B were created by the researcher. The first part required the participant connect numbers 1 to 50 randomly positioned on A4 paper by a pencil line. In the second part participants were asked to connect between numbers (25 numbers) and Thai letters (25 letters) maintaining accuracy and sequential order (1, ก, 2, ข, 3, ฃ...). The participant was given 45 seconds to complete each part and scored by correct connected letters in TMT A or number and letter in TMT B. The first set was used as practice and 2 alternative versions were used as pre-test and post-test tasks equally.

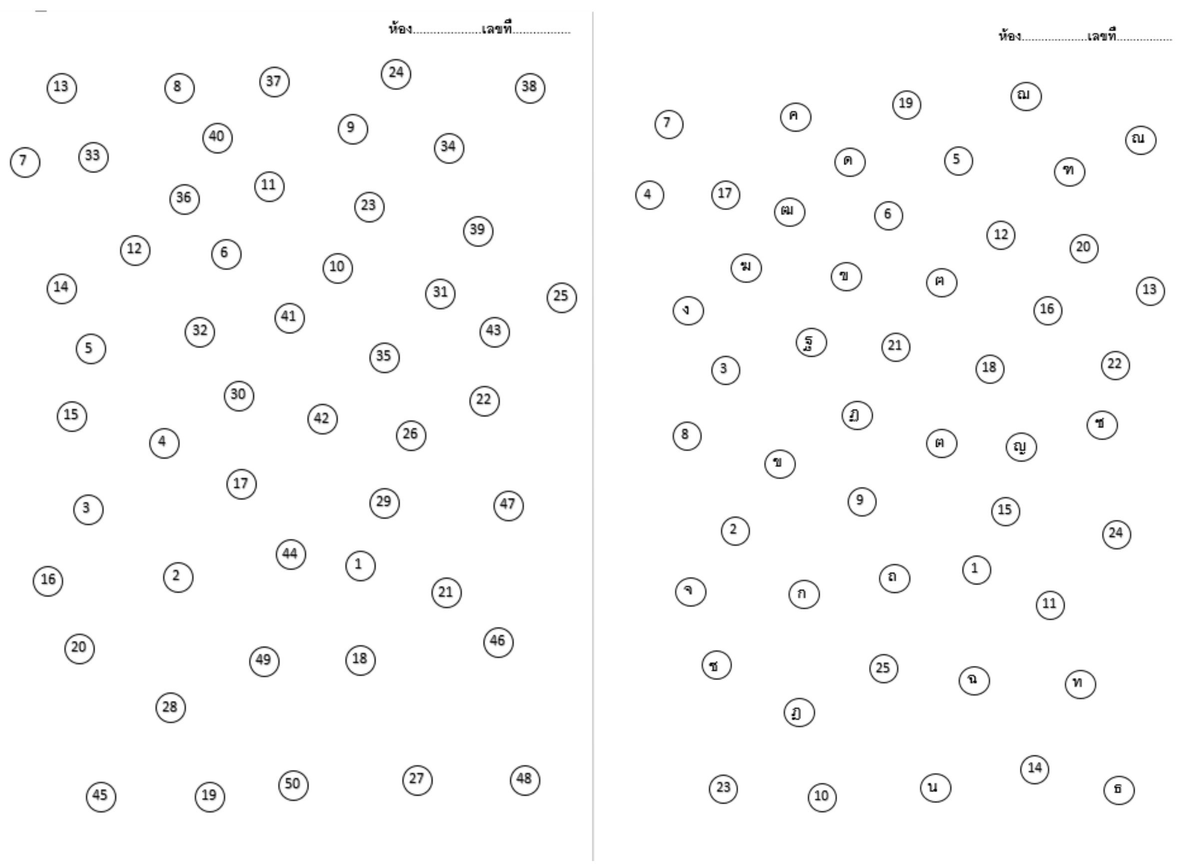

Figure 5-2 Example of TMT A (left) and TMT B (right)

5.2.3.3 Colour-Word Interference Test

The colour-word interference Stroop test was part of the D-KEFS test, with an aim to measure the performance of inhibited a dominant and automatic responses (Chaytor et al., 2006; Homack et al., 2005). In this study, an adapted version of the Stroop colour and word test was employed. The test consisted of three types of words. The first type was a colour word printed in the same ink (red printed in red ink), which measured congruence. The second type consisted of neutral trials with Xs printed in different coloured inks and requiring participants to name the colour of ink. The third type was colour words printed in different ink colours that required participants to name the colour of ink (e.g., the word “red” is printed in green colour and the participant must name “green”). The colour word (interference effect) was used to measure the executive function (index of inhibition). There were 72 words in total which 24 congruent words 24 neutral words and 24 interference words. To complete this, participants were given a 90 seconds time limit to write down the correct answer under the word provided. Three alternative Thai versions were designed and alternated across practice, pre-test and post-test phases Therefore, participants were scored for each of neutral, congruent and incongruent trail as by the number of items correctly completed.

ห้อง....................เลขที่.................

Xs	เขียว	Xs	แดง	ชมพู	น้ำเงิน
เขียว	ม่วง	ม่วง	Xs	เหลือง	Xs
ม่วง	Xs	Xs	แดง	น้ำเงิน	เขียว
Xs	น้ำเงิน	ม่วง	เขียว	Xs	เขียว
ชมพู	Xs	น้ำเงิน	Xs	เหลือง	ม่วง
น้ำเงิน	ม่วง	Xs	แดง	ชมพู	Xs
Xs	Xs	ชมพู	ชมพู	แดง	น้ำเงิน
เขียว	ชมพู	แดง	Xs	แดง	Xs
Xs	น้ำเงิน	ม่วง	Xs	ม่วง	ชมพู
ม่วง	เหลือง	ชมพู	Xs	แดง	Xs
เขียว	น้ำเงิน	Xs	ชมพู	Xs	เหลือง
เขียว	Xs	เขียว	น้ำเงิน	ชมพู	Xs

Figure 5-3 Sample of Stroop task.

5.2.3.4 Verbal Fluency Test

The original Verbal Fluency test was designed to measure letter fluency, category fluency and category switching. In this study the test was adjusted to a classroom environment by requiring participants to write as many words as they could. The words began with 3 Thai letters and participant was given 60 seconds to complete each letter. Therefore, participants were scored by the number of words produced from across the three trials to measure the executive function.

5.2.4 Other measures

5.2.4.1 O-Net test

At the end of the academic year, as described in Chapter 2 all grade 6 children completed the Thai Ordinary National Educational Test (O-NET). The O-Net test was marked independently by a public organisation, NIETS (National institute of Educational Testing Service (Public organization). Overall scores and individual subject-specific scores for each pupil were returned to the school and were used in my study as a measure of academic attainment.

5.2.4.2 Positive and Negative Affect Scale (PANAS)

The PANAS, developed by Watson, Clark and Tellegen (D. Watson & Clark, 1988), is a 20 item self –report checklist to investigate positive (e.g. interested, excited, pround) and negative affect (e.g. distressed, upset, guilty). The 10 positive items reflect an individual experiences pleasurable engagement with the environment, whereas the 10 negative items indicate subjective distress and un-pleasurable engagement. A paper and pencil Thai language version of the task was created using the same descriptors in the English language version.

For each item, at baseline and post-intervention, participants were asked to complete the PANAS assessing their mood by writing down the number that correlated to how they felt at the present moment on a 5-point Likert scale (1= very slightly/not at all to 5= extremely). The scores for the 10 words relating to positive emotions, and 10 words relating to negative emotions, were added together to give an overall measure of a participant's positive affect (PA) and negative affect (NA). An example of the PNS can be found in Appendix C.

5.2.4.3 Semi-quantitative Food frequency questionnaire

The first part of the questionnaire asked participants to complete their demographic status such as age, gender, number of family members, family income, height and weight. Additionally, participants were asked whether they regularly consumed any food supplements (i.e. multi-vitamin supplements).

In the second part of the questionnaire, typical food consumption was assessed using a food frequency questionnaire (FFQ). This FFQ was adapted for use in Thailand by the researcher and included reference to typical foods found in Thailand. The FFQ consisted of 23 items of food, which consisted of 3 categories of carbohydrate, 10 categories of protein, 5 categories of fruit and vegetables, and 5 categories of snack (a copy of the FFQ can be found in Appendix D). The FFQ was completed 1 week before the experiment started, a week in the middle of the intervention phase and then during the last week of the intervention. Analysis of these diaries allowed us to check the basic diets of participants and to note any changes in food selection over the course of the experiment.

5.2.4.4 Daily record

Students were also asked to keep a daily food record to assess foods consumed during the SLP. In the daily record students were instructed to select the appropriate column that reflects their consumption (consumed in total, partially eaten and not eaten at all) for main meals and intervention fruits. Analysis of these data allowed us to calculate an estimate of the consumption of the flavonoid-rich or flavonoid-poor fruit daily (see Appendix E).

5.2.5 Procedure

Participants were introduced to general information about the study, its purpose and objectives. They were also asked to complete a consent form via their parent/guardian, which was then brought back to their school teacher after signed.

On the practice day, participants were all seated in the school hall and given instructions with an opportunity to practice for each task to avoid the practice effect as follows;

DSST (90 secs)

TMT (90 secs, 45 secs per test)

Stroop (90 secs)

Verbal Fluency (180 secs, 60 secs per letter)

On the test day, school teacher allowed me to test participants out of their normal classroom during day, participants were seated in a classroom to complete the measures as below:

Cognitive tasks

DSST (90 secs) took approximately 5 minutes.

TMT (90 secs, 45 secs per test) took approximately 5 minutes.

Stroop (90 secs) took approximately 5 minutes.

Verbal Fluency (180 secs, 60 secs per letter) took approximately 5 minutes.

Positive and negative affect schedule (PANAS) took approximately 5 minutes.

Self-report semi quantitative FFQ took approximately 10 minutes.

To complete every measure took approximately 35 minutes.

During 12 weeks of the intervention period students consumed their habitual meals and were given the intervention fruits by class teacher in school lunchtime. The participants were also asked to keep a daily food record which was collected weekly throughout the length of intervention.

The intervention ended by the last Friday of January (week 12 of the intervention) and the participants completed the O-Net score by Saturday. The cognitive tests were tested in the same order as at baseline in the week after the end of intervention.

5.3 Analysis

5.3.1 Data Clean-Up

Of the 404 participants recruited to the study, 46 participants failed to consume at least 50% of their intervention across the 12 weeks of the trial and were subsequently excluded from the analyses. In total, data from 183 HF participants and 175 LF participants were analysed.

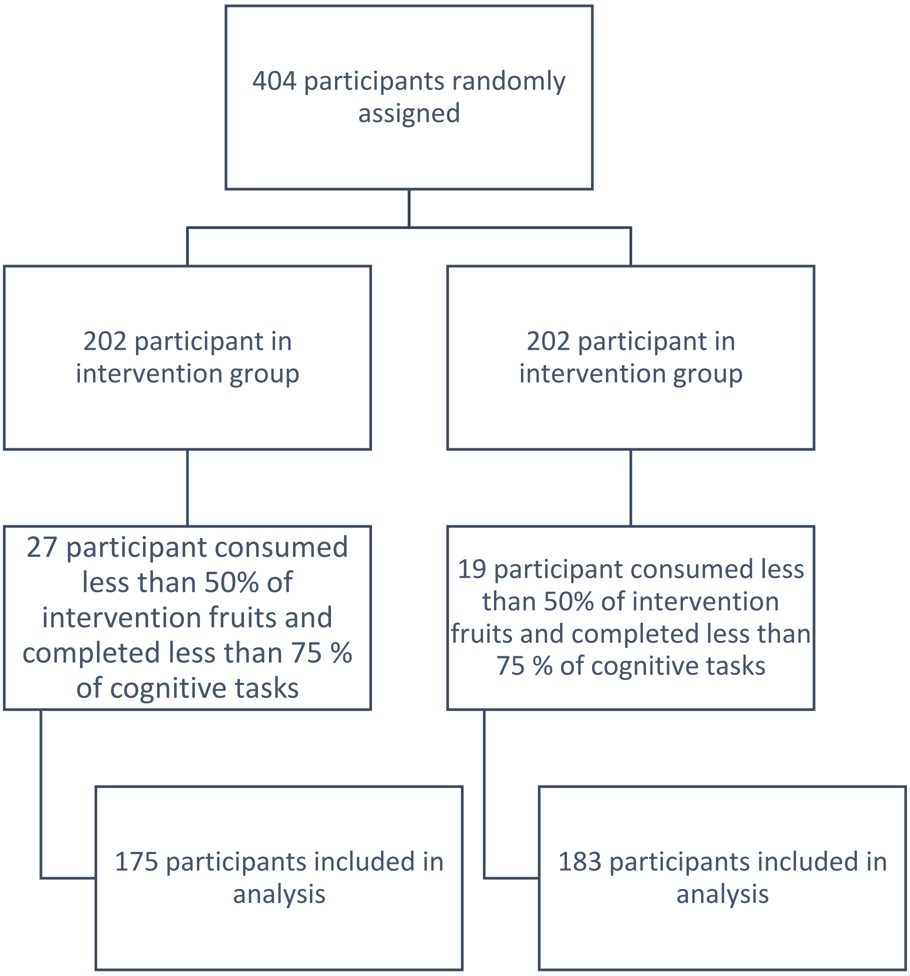

Figure 5-4 Diagram of data clean-up

5.3.2 FFQ

For each food type, the number of portions consumed was multiplied by the frequency of consumption per week. This value was then divided by 7 to calculate the average number of portions consumed per day.

5.3.3 Flavonoids content from intervention fruits

Flavonoids content from the intervention fruits, which have come from USDA data (orange, papaya, water melon, melon and banana) and the Thai publication (guava, pineapple and pomelo), was calculated from the daily food record. The record of how much of the intervention was consumed (1 = consumed in total, 0.5 = partially eaten, 0 =not eaten at all) was then multiplied by the flavonoid content of the intervention (i.e. per 40g portion) on that particular day. This was repeated for all days of the intervention period, added together and divided by the number of days in the intervention period to average flavonoid intake per day.

5.3.4 High and low scores

In general, higher scores and positive scores of each variable indicate better performance. In contrast, the composite score of executive function, lower scores and negative increases over time indicate better performance.

5.3.5 O-NET performance

The 8 subjects of O-Net score including Thai language, Mathematics, Science, Social Studies, Religion and Culture, Health Study, Physical Education, Arts, Occupations and Technology, and Foreign languages were marked independently by NIETS (National institute of Educational testing Service to yield a percentage score. The individual subject-specific scores for each participant were returned to the school and were used in my study as a measure of academic attainment by generating a proportion score for each subject.

5.3.6 Individual task performance

The proportion individual score of the cognitive tasks were calculated as below:

TMT task, TMT A and B were score by corrected to connect between numbers (50 numbers) (TMT A) and numbers (25 numbers) and Thai letters (25 letters) (TMT B) maintaining accuracy and sequential order and generate a proportion score to represent the score of TMT.

Correct responses to Stroop task congruent and incongruent trials were calculated to generate a Stroop proportion score.

The total number of words write down to the Verbal Fluency answer sheet was represented the VF score.

5.3.7 Composite score calculation

In terms of a composite score of executive function, I calculated the mean of z-scores for the proportion score of TMT, Stroop and z-score for verbal fluency.

5.3.8 Statistical Analysis Plan

SPSS version 22.0.0 was used to analysis the data of this thesis. A 2x2 Repeated Measures Analysis of variance (ANOVA) with 2 flavonoid treatments (2 groups: HF or LF) and 2 time points (2 time points: pre- and post-intervention) were used to detect the effect of flavonoids between treatments and over time changes for each of the cognitive tasks. This was followed, where appropriate, by Bonferroni comparisons. Changes in O-NET score were assessed by independent T-test.

5.4 Results

5.4.1 Demographics

Descriptive statistics for baseline demographic characteristic of participant are shown in Table 5-1.

Participant were aged between 10 to 13 years old, with a mean age of 11.70 (SD=0.52). There were 166 males (HF=83; LF=83) and 192 females (HF=92; LF=100). Weight ranged from 21 to 102 kgs, with a mean of 43.92 (SD=12.96), being higher in the LF group (M=44.07, SD=13.11) than HF (M=43.67, SD=11.79). Height of participants ranged from 115 to 175 cms (M=151.78, SD=8.32), with HF group (M=151.57, SD=7.80) being well matched to the LF group (M=151.97, SD=8.86).

Family income in HF group was higher than 30,000 TB per month in 21% of the group and also 25% of the LF group. Number of family members ranged from 2 to 15 people, with 5.05 (SD=2.08) in HF group and 5.15 SD=2.02) in LF. Grade point average of grade 5 ranged from 1.55 to 4.00 (M=3.06, SD=0.64), being numerically higher in the HF group (M=3.06, SD= 0.60) than LF (M=3.05, SD=0.88).

Table 5-1 Baseline demographic characteristic of participants.

Baseline Characteristic	HF Mean (SD) (n =175)	LF Mean (SD) (n =183)	Total Min (n =358)	Total Max (n =358)	Total Mean (SD) (n =358)
Age (years of age)	11.07(0.53)	11.69(0.52)	10	13	11.70(0.52)
Gender:					
Females	92	100	-	-	166
Males	83	83	-	-	192
Anthropometrics:					
Weight (kg)	43.67(11.79)	44.07(13.11)	21	102	43.92(12.56)
Height (cm)	151.57(7.80)	151.97(8.86)	115	175	151.78(8.32)

Baseline Characteristic	HF Mean (SD) (n =175)	LF Mean (SD) (n =183)	Total Min (n =358)	Total Max (n =358)	Total Mean (SD) (n =358)
% Family income (TB/M)					
0-5,000	6.86	4.92	-	-	5.78
5,001-10,000	18.29	16.94	-	-	17.60
10,001-15,000	14.86	15.85	-	-	15.36
15,001-20,000	18.86	14.21	-	-	16.48
20,001-25,000	8.00	9.29	-	-	8.66
25,001-30,000	10.29	12.02	-	-	11.17
More than 30,000	21.14	24.59	-	-	22.91

Baseline Characteristic	HF Mean (SD) (n =175)	LF Mean (SD) (n =183)	Total Min (n =358)	Total Max (n =358)	Total Mean (SD) (n =358)
Number of family members	5.05(2.08)	5.15(2.02)	2	15	5.09(2.03)
Grade Point Average From Grade 5	3.06(0.60)	3.05(0.68)	1.55	4.00	3.06(0.64)

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; TB/M= Thai Baht per month.

5.4.2 Daily Food record and Food Frequency Questionnaire

The total amount of flavonoids from intervention fruit consumption was 7,008.32 µg per day (SD=6,113.89), with HF and LF averaging 13,131.51 µg per day (SD=1,152.81) and 1,152.81 µg per day (SD=133.27) respectively shown in Figure 5-5.

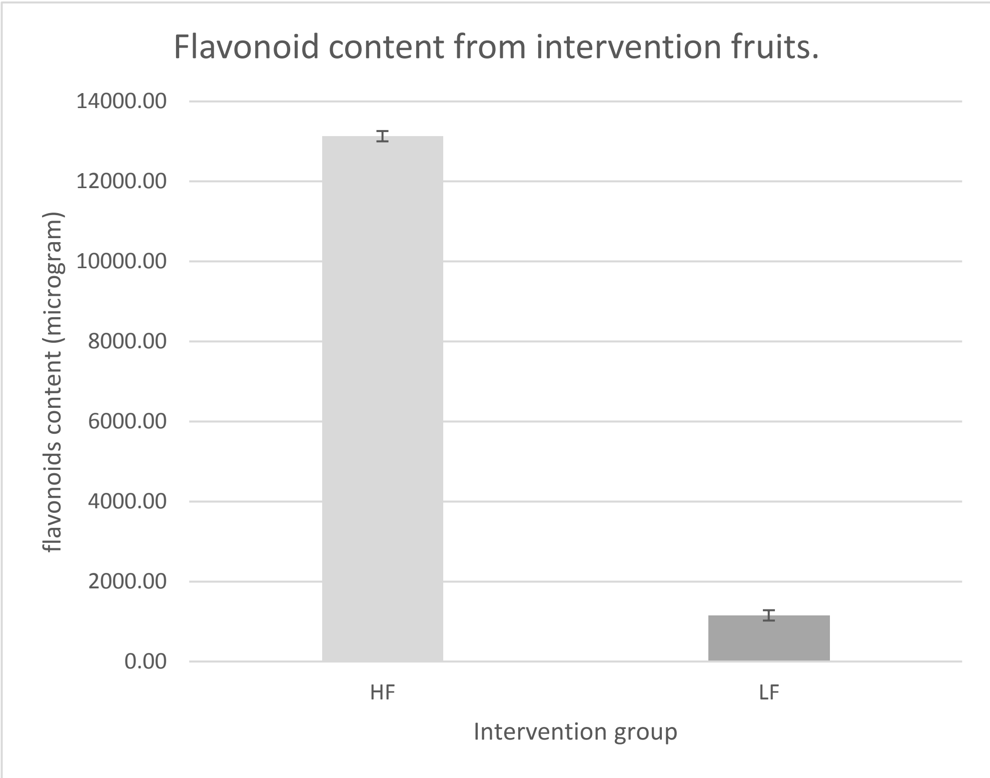

Figure 5-5 Mean and standard error of flavonoids content from intervention fruit.

Data from Food Frequency Questionnaire revealed that the average numbers of the food portion consumed of each food group shown in Table 5-2. The independent t-test revealed that there was no statistical difference at baseline (FFQ 1) and at the end of intervention (FFQ3). However, there were significant higher intake of LF participants than HF participants including carbohydrate (HF=6.66; LF=7.67) (t (356) = -2.668, *p* =.008), milk (HF=1.50; LF=1.75) (t (356) = -2.132, *p* =.034), vegetable (HF=2.27; LF=2.96) (t (356) = -2.490, *p* =.013), Fruit (HF=2.58; LF=3.13) (t (356) = -2.219, *p* =.027), and soft drink (HF=0.85; LF=1.17) (*t* (356) = -2.668, *p* =.008).

As can be seen in Table 5-3, the analysis of fruit, vegetable, and fruit and vegetable consumption have shown that there was no statistical difference in the consumption of vegetables alone (*F* (2,356) =2.110, *p*=.147). However, in fruit alone and fruit and vegetables combined there was a significant difference of the main effect of group (*F* (2,356) =5.121, *p*=.024) and (*F* (2,356) =5.214, *p*=.023) respectively. There were no significant interactions.

Table 5-2 Portion of food consumption from Food Frequency Questionnaire of participants.

	HF Mean(SD)	LF Mean(SD)	*t*	*p*
Food Frequency Questionnaire :				
Carbohydrate Food Group;				
1stFFQ	7.14(3.38)	7.24 (3.51)	-0.280	.780
2ndFFQ	6.66(3.58)	7.67(3.56)	-2.668	.008*
3rdFFQ	6.69(4.10)	6.44(3.63)	0.621	.535
Meat and Protein Food Group				
Meat				
1stFFQ	8.94(6.42)	8.89(6.61)	0.082	.935
2ndFFQ	7.41(5.63)	8.37(5.46)	-1.635	.103
3rdFFQ	6.66(5.06)	6.82(4.38)	-0.317	.751
Milk				
1stFFQ	2.11(1.92)	2.10(1.84)	0.070	.944
2ndFFQ	1.50(1.08)	1.75(1.10)	-2.132	.034*
3rdFFQ	1.84(1.32)	2.01(1.39)	-1.247	.213

	HF Mean(SD)	LF Mean(SD)	*t*	*p*
Soy				
1stFFQ	1.37(1.99)	1.29(1.35)	0.431	.666
2ndFFQ	1.08(1.60)	1.20(1.36)	-0.756	.450
3rdFFQ	1.01(1.24)	1.17(1.24)	-1.224	.222
Egg and Bean				
1stFFQ	2.36(1.64)	2.43(1.80)	-0.402	.688
2ndFFQ	1.98(1.52)	2.17(1.55)	-1.164	.245
3rdFFQ	1.93(1.30)	2.08(1.93)	-1.066	.287
Fruits and Vegetable Food Group				
Vegetable				
1stFFQ	2.90(3.14)	3.09(2.91)	-0.591	.555
2ndFFQ	2.27(1.97)	2.96(3.14)	-2.490	.013*
3rdFFQ	2.61(2.51)	2.56(2.30)	0.196	.844

	HF Mean(SD)	LF Mean(SD)	*t*	*p*
Fruit				
1stFFQ	3.39(2.49)	3.84(3.21)	-1.471	.142
2ndFFQ	2.58(2.20)	3.13(2.46)	-2.219	.027*
3rdFFQ	3.05(2.74)	3.14(2.43)	-0.335	.738
Juice and snack Food Group				
Juice				
1stFFQ	0.83(1.01)	0.74(0.66)	1.046	.296
2ndFFQ	0.59(0.63)	0.67(0.63)	-1.128	.260
3rdFFQ	0.67(0.75)	0.72(0.75)	-0.642	.521
Snack and Dessert				
1stFFQ	2.10(1.51)	1.93(1.68)	0.981	.327
2ndFFQ	1.79(1.48)	2.09(1.48)	-1.959	.051
3rdFFQ	1.75(1.38)	1.90(1.48)	-0.972	.331

	HF Mean(SD)	LF Mean(SD)	*t*	*p*
Soft drink				
1stFFQ	1.10(0.94)	1.05(1.01)	0.161	.872
2ndFFQ	0.85(0.81)	1.17(1.10)	-3.226	.001*
3rdFFQ	0.90 (0.87)	1.10(1.11)	-1.781	.076
Candy				
1stFFQ	0.64(0.79)	0.64(0.88)	0.001	.999
2ndFFQ	0.51(0.75)	0.55(0.62)	-0.656	.512
3rdFFQ	0.53(0.69)	0.59(1.02)	-0.676	.500

Table 5-3 Mean and standard deviation of fruit, vegetable and fruit and vegetable consumption in portion from semi-quantitative food frequency questionnaire.

Group	FFQ 1 Mean(portion) Sd	FFQ 2 Mean(portion) Sd	FFQ 3 Mean(portion) Sd	*F*	*p*
FV					
1(HF)	6.29(4.55)	4.85(3.58)	5.66(3.97)	T:11.407	.000*
2(LF)	6.93(4.80)	6.09(4.25)	5.70(3.54)	G:3.865	.050*
				TXG:2.800	.065
V					
1(HF)	2.90(3.14)	2.27(1.97)	2.61(2.51)	T:3.373	.035*
2(LF)	3.10(3.91)	2.96(3.14)	2.56(2.30)	G:1.917	.167
				TXG:2.327	.098
F					
1(HF)	3.39(2.49)	2.58(2.20)	3.05(2.47)	T:11.996	.000*
2(LF)	3.84(3.21)	3.13(2.46)	3.14(2.43)	G:3.290	.071
				TXG:1.157	.315

5.4.3 O-NET score

Similar to the previous experiments, O-Net scores were used to investigate the effects of flavonoid supplementation on real life academic attainment. The mean proportion score of the O-NET in both groups was nearly identical (0.502 in HF and 0.503 in LF group). To compare the O-Net score (proportion score of 5 subjects) at the end of intervention, a T-test analysis was used. The output was consistent with the last two experiments, in showing that no significant differences were found between HF and LF conditions (*t* (365) =-0.091, *p*=.928).

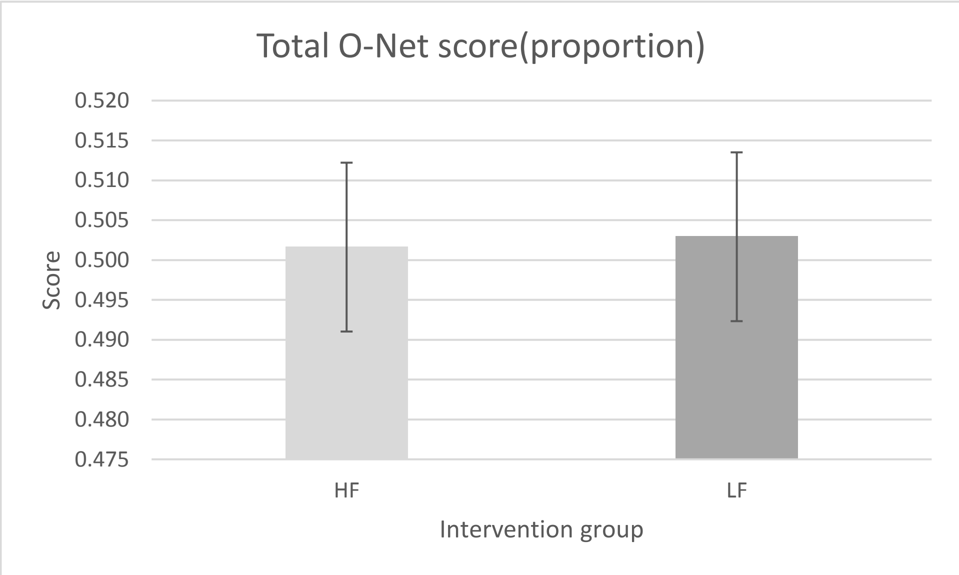

Figure 5-6 Mean and standard error for proportion of total 5 subject of O-Net score in all groups of participants.

5.4.4 Composite score of executive function

Both HF and LF performed better over the course of intervention time with the HF group showing better performance than LF at the end of intervention (HF=-0.06 versus LF=-0.02). However, there was again a statistical difference in terms of the effect of time (F (1,356) =5.035, p=.025), but failed to reach significance on a main effect of group (F (1,356) =0.323, p=.570), or a time x group interaction (F (1,356) =0.012, p=.914) respectively.

The results obtained from executive function analysis are shown in Figure 5-7.

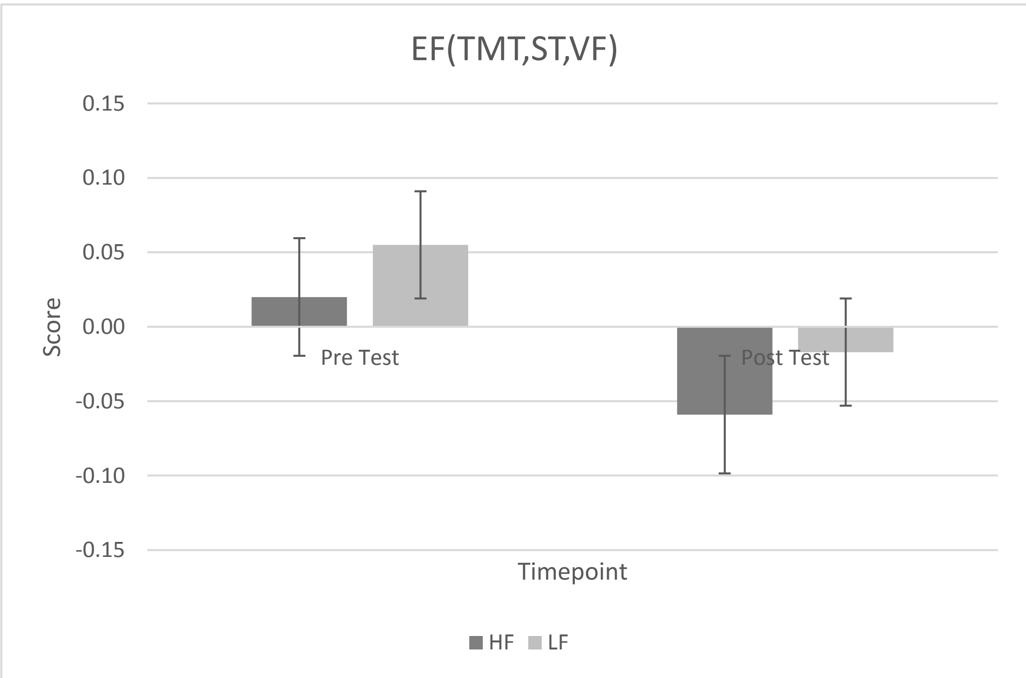

Figure 5-7 Composite score of Executive function (±standard error of the mean)

5.4.5 PANAS Mood

In terms of positive affect scores, the HF condition had slightly more positive scores than the LF condition at both baseline and post-intervention, although both groups became marginally less positive over the duration of the intervention, but with less of a drop in the HF group (35.49 and 35.37) than the LF group (35.95 and 35.57). In order to measure the effects of the intervention, repeated-measures ANOVAs were used. There were no significant differences in terms of main effects of time (F (1,356) =0.663, p=.427), group (F (1,356) =0.301, p=.584), or time x group interactions (F (1,356) =0.185, p=.667).

The average scores for negative affect, at baseline that was higher for HF participants were higher scores than LF participants (HF=23.60 versus LF=23.05) and at the end of the intervention HF participants negative affect was increased (indicated more negative affect) and still higher than LF participants (HF=23.81 versus LF=23.00). As can be seen in Table 5-4, there was no statistic significant in the main effect of time (F (1,356) =0.053, p=.818), group (F (1,356) =0.959, p=.328), or time x group interaction (F (1,356) =0.122, p=.727).

Table 5-4 Composite score of cognitive and Mood Measures: Z score, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all participants.

Cognitive/Mood Variable	Time	HF Mean Z (SD)	LF Mean Z (SD)	Repeated Measures ANOVA *F*	 *p*
PANAS					
Positive affect	Baseline	35.49(6.23)	35.95(6.29)	T:0.663	.427
	Post-Intervention	35.37(6.35)	35.57(6.75)	G:0.301	.584
				TXG:0.185	.667
Negative affect	Baseline	23.60(7.45)	23.05(7.62)	T:0.053	.818
	Post-Intervention	23.81(7.04)	23.00(7.29)	G:0.959	.328
				TXG:0.122	.727

Cognitive/Mood Variable	Time	HF	LF	Repeated Measures ANOVA	
		Mean Z (SD)	Mean Z (SD)	*F*	*p*
O-Net Score		0.502(0.14)	0.503(0.14)	T(365) =-0.091	.928

5.4.6 Individual cognitive task results

Table 5-5 (below) shows the mean individual performances on the TMT, Stroop and VF for both HF and LF groups.

5.4.6.1 Symbol Digit Modalities Test

In the HF condition, participants had a higher score on the digit symbol substitution task at baseline (HF=58.06 versus LF=57.62) and again were better at post intervention (HF=62.36 versus LF=60.24) than LF condition. Both groups were better over time as indicated by the significant main effect of time (F (1,356) =22.577, p<.001), but there was no main effect of flavonoids condition (F (1,356) =1.143, p=.286) or a main effect of time x flavonoids treatment interaction (F (1,356) =1.486, p=.224).

5.4.6.2 Trail Making Test

The proportion score for TMT A and B was shown to be very slightly higher in the LF condition at baseline (LF=0.36 versus HF=0.35) but identical at the end of the study (LF=0.35 versus HF=0.35). This task failed to detect the difference effect between the two flavonoids conditions. Results from repeated ANOVA confirmed this, no main effect of time (F (1,356) =2.898, p=.085), no main effect of flavonoids condition (F (1,356) =0.771, p=.380) or a main effect of time x flavonoids treatment interaction (F (1,356) =0.009, p=.924).

5.4.6.3 Colour-Word Interference Test

In the HF condition participants performed slightly less accurately at baseline (HF=0.44 versus LF=0.45) and correspondingly so post intervention (HF=0.46 versus LF=0.48) than the LF participants. Both groups were better over time as indicated by the significant main effect of time (F (1,356) =16.973, p<.001), but there was no main effect of flavonoids condition (F (1,356) =2.260, p=.134) or a main effect of time x group interaction (F (1,356) =0.609, p=.436).

5.4.6.4 Verbal Fluency Test

In the HF condition participants performed better on the verbal fluency task at baseline (HF=38.14 words versus LF= 37.63 words) and numerically again so post intervention (HF=34.41 words versus LF =34.28 words) than LF participants. Both groups were worse over time as indicated by the main effect of time (F (1,356) =48.639, p<.001) but there was no main effect of flavonoid condition (F (1,356) =0.109, p=.742) or a time x flavonoids treatment interaction (F (1,356) =0.145, p=.703).

Table 5-5 Individual score of cognitive task, Standard Deviation and Repeated Measures ANOVA Statistics for Time by Group for all participants.

Cognitive/Mood Variable	Time	HF Mean (SD)	LF Mean (SD)	*F*	*p*
Processing Speed:	Baseline				
DSST	Post-Intervention	58.06(11.50)	57.62(11.92)	T:22.577	.000*
		62.36(14.47)	60.24(13.88)	G: 1.143	.286
				TxG: 1.486	.224
TMT Proportion	Baseline	0.35(0.07)	0.36(0.08)	T:2.898	.085
	Post-Intervention	0.35(0.07)	0.35(0.08)	G: 0.771	.380
				TxG: 0.009	.924

Cognitive/Mood Variable	Time	HF Mean (SD)	LF Mean (SD)	*F*	*p*
ST Proportion	Baseline	0.44(0.10)	0.45(0.11)	T:16.973	.000*
	Post-Intervention	0.46(0.11)	0.48(0.12)	G: 2.260	.134
				TxG: 0.609	.436
Verbal Fluency	Baseline	38.14(10.44)	37.63(9.98)	T:48.639	.000*
	Post-Intervention	34.41(10.75)	34.28(9.82)	G:0.109	.742
				TXG:0.145	.703

Note: HF = high flavonoids group; LF = low flavonoids group; SD = standard deviation; n = number of participants; T= Time; G= Flavonoids treatment group; TxG= time x Flavonoids treatment group interaction. *p<.05.

5.5 Discussion

In this chapter, we sought to clarify the effect of 80 grams rich flavonoids fruit on the EF and mood, in an attempt to replicate the pattern of effects found in Chapter 3. The EF tasks including DSST, TMT, Stroop and Verbal fluency were administrated to measure processing speed, flexibility of thinking, inhibition and fluency. Disappointingly, the results did not reveal any evidence to confirm our previous finding in Chapter 3. DSST was used to measure processing speed which may underpin executive and memory process but the finding of our current study was again unable to detect an effect of additional flavonoid based fruit consumption on cognitive performance. The composite score of EF (mean z score of TMT proportion, Stroop proportion and VF), revealed that both HF and LF conditions performed better over time but analysis of the relationship showed no between condition differences.

An analysis of individual TMT performance revealed no effects of flavonoid consumption on accuracy. In contrast, Crews Jr et al. (2008b) found that 6 weeks of either flavonoids- and procyanidine dark chocolate affected cognitive ability in older adult although they also found that TMT task produced no significant differences between the two groups. This effect though is inconsistent with the finding from Mastroiacovo et al. (2015) who conducted a cocoa drink supplementation in older adults during an 8 week intervention and demonstrated a significant quicker completion time for both TMT A and B. The study of Mastroiacovo et al. (2015) also showed that performance in verbal fluency in their HF group was greater than an intermediate group and LF group. However, this effect of flavonoids on verbal fluency was not shown in the current study. In terms of Stroop performance, in the current chapter, again as for other EF tasks, we were unable to detect any differences between the two conditions as a function of the intervention. Interestingly, Crews Jr et al. (2008b) also reported no effects on a version of the Stroop task.

In terms of O-Net performance, consistent with Chapters 3 and 4, we were unable to detect any effects of flavonoid intake on academic attainment. To date, there are no evidence to support the effect of flavonoids intake on standard measures of academic performance. As discussion in Chapter 4, Eilander et al. (2010), however reviewed the literature of multiple micronutrient on cognitive performance in school age children, demonstrating that a marginal increase in fluid intelligence and academic performance may be related to multiple micronutrient supplementation. Thus, to improve academic outcome in schoolchildren it may be that more complex multiple micronutrient supplements are required rather than focusing on single groups such as flavonoids.

In terms of mood, there were no significant differences between the HF and LF conditions, in contrast to the mood affects found in Chapter 3. However, this failure to replicate is consistent with the finding of Chapter 4 and is also consistent with Kean et al. (2015) who demonstrated no significant difference in positive or negative affect from supplementation of a high flavanone 100 % orange juice containing 305 mg flavanone during an 8 weeks intervention in older adults.

One consideration in interpreting this result was that there was a postponement in administering the O-Net test in 2015, with the test normally taking place at the end of January but this year being delayed until the end of February. Our intervention ended by January and the students were then tested on the cognitive tasks and mood scale. However, the O-Net test was not administered until a month later, such that it is feasible that the absence of any direct effect from flavonoids supplementation could be because the effect had dissipated by this point.

Chapter 6 Discussion

6.1 Introduction (overview of the studies)

A number of studies from human epidemiological and intervention RCTs have demonstrated that flavonoids may produce increases in cognitive performance. A number of different mechanisms of action may underlie the boost in cognitive function but it is clear, from a number of cellular and molecular studies, that flavonoids can improve neuronal connectivity and protect against neurodegeneration. The literature supporting such effects in children is more limited, although evidence of such effects in this age group would have significant potential implications for learning and educational performance. In the current thesis, I hypothesised that the effects of chronic flavonoid supplementation in adults would be replicated in a sample of children aged 11-13 years old. Additionally, I anticipated that supplementation of flavonoid-rich fruit over a single school semester would show clear improvements in cognitive performance (primarily episodic memory and executive function), and furthermore, the improvement of cognitive performance would be associated with better academic attainment at the end of the school year. In this Chapter, I will summarise the findings of my studies and place them into context with previous literature in this field. I will also discuss the limitations of my studies and future directions for this work.

6.2 Summary of findings

The first study (Chapter 3) investigated the effects of chronic flavonoid supplementation on cognition, mood and academic attainment. Participants were randomly allocated to one of two conditions consuming a 40 gram portion of fruit either rich in flavonoids (average 6,245.15 µg per day) or low in flavonoids (average 227.80 µg per day) for 12 weeks. Cognitive performance was measured at baseline or at the end of the supplementation period using a battery of cognitive tasks: Auditory Verbal Learning Task (AVLT) to assess verbal episodic memory, flanker and letter memory tasks to measure executive function, the Digit Symbol Substitution Task (DSST) to assess processing speed and Positive and Negative Affect Scales to measure changes in mood. Additionally, academic attainment of the children was measured using the nationwide O-NET score at the end of intervention. The analysis of cognitive tasks revealed a positive effect on a composite score of executive function and negative effect on negative mood affect following HF intake that is considered further in sections 6.2.1, 6.2.2, 6.2.3, and 6.2.4. With respect to O-NET score, the main outcome of my study, I failed to detect an influence of either HF or LF on performance, suggesting that the O-NET measure was not adequate to demonstrate subtle differences and changes in effects following chronic flavonoid fruit intervention, or alternatively that the O-NET may not influenced by consumption of flavonoids in fruit. Despite the absence of an effect on O-NET scores, the apparent sensitivity to executive function in Chapter 3 lead me to probe the nature and reliability of this effect in Chapter 4.

Given the positive effects of our flavonoid-rich fruit intervention on executive function seen in Chapter 3, the next step was to attempt to replicate and extend my findings (Chapter 4). Here, in my second study, the battery of tasks was focussed more selectively on executive function, the dose of flavonoids consumed per day was significantly increased and an additional group introduced to control for intake (to tease apart the benefits of flavonoid intake from the more general (potential) benefits of rich flavonoids fruit). In this study, participants were randomly allocated to one of three conditions: control (no fruit but biscuit or bun supplementation), 80 gram low flavonoid (average flavonoid content of 1,032.83 µg per day) and 80 gram high flavonoid group (average flavonoid content of 13,257.70 µg per day). Participants were supplemented for 7 weeks (due to unanticipated difficulties in gaining access to the school) in this study and the cognitive battery was performed at baseline and the end of the intervention. A more extensive executive function battery was employed which included the flanker task, letter memory task and StopGo task, again mood was assessed at the beginning and end of the intervention and O-NET score was assessed at the end. Unfortunately, the positive effects that appeared in Chapter 3 were not replicated which with no significant effects of HF versus LF or control conditions on executive function, mood, and O-NET score. These results are further considered in section 6.2.2, 6.2.4, and 6.2.5.

For my final study, the discrepancies in findings between study 1 and 2 were explored further. Here, I explored the effects of flavonoid supplementation on executive function, mood and academic outcome in a much larger number of participants (power=0.99). To ensure that the required numbers of participants could be tested, I developed pen and pencil cognitive tasks that could be completed in a classroom context. As usual, cognitive batteries and mood scale were investigated at beginning and end of a 12 week intervention. In this experiment, participants were randomly allocated to receive either an 80 gram portion of high flavonoid fruit (average flavonoid content 13,131.51 µg per day) or low flavonoid fruit (average flavonoid content of 1,152.81 µg per day). Unfortunately, no significant differences between groups were seen similar too Chapter 4, with no significant effects of HF supplementation on cognitive performance, mood affect, and O-NET scores. These findings are further considered in section 6.2.2, 6.2.3, 6.2.4, and 6.2.5 below.

6.2.1 Memory

The Thai version of the Auditory Verbal Learning task (AVLT) was used to measure verbal episodic memory in Chapter 3. An English language version of this task was previously used by Whyte et al. (2015), who found that acute wild blueberry supplementation in 7-10 year olds children was associated with improved delayed word recall following 127 mg and 253 mg anthocyanin treatment. Similarly, Kent et al (2015) also found a positive effect of flavonoid supplementation of AVLT task performance following an anthocyanin-rich cherry juice administered to older adult with mild to moderate dementia for 12 weeks. In term of the individual scores of total acquisition in Chapter 3, which is a measure of the total number of words recalled for the first 5 trials presenting total number of word learned, no main effects of time or treatment were found. Similarly, measures of delayed recall, which is the number of words recalled after a 25 minute delay, revealed no main effect of time or treatment, but an unexpected time by treatment was significant with participants receiving LF performing better than HF. However, when scores for total acquisition and delayed recall were combined as a composite score of memory function, no main effect of time, group, and time X group were observed. It remains possible that a Thai language version of this task was not sensitive enough to detect any changes in this domain following long-term flavonoids intervention. The limited of association of memory function measured by this task following flavonoids supplementation may have been due to the lack of standard Thai language version used in Thai at the time of the study. More sensitive and more reliable of this task in Thai language need to develop to address the limitations of the task left by this research. Additionally, motivational factors may also have affected performance on this task and were not measured. To sum up the result in Chapter 3 failed to replicate the positive effect observed by Whyte et al. (2015) and do not support the evidence that verbal episodic memory may be enhanced by flavonoids.

6.2.2 Executive function

In Chapter 3 the flanker task, a measure of attention-related executive function, and letter memory, a measure of updating, were used to demonstrate effects of flavonoids on the executive function domain after supplementation of 40 grams fruits. As an individual cognitive task, letter memory response time and flanker interference effect were calculated. For the letter memory task, a significant effects of time was seen, but not group or group X time interactions, suggesting a possible practice effect for this task. There are possibility that the influence of flavonoids on letter memory might vary according to the difficulty of the task. Data were then analysed separately for both easy trial (5 letter), medium trial (7 letter) and hard trial (9 letter). However, this failed to reach significance of treatment in 5 letter (F (1,159) = 1.411, p = .261), 7 letter (F (1,159) = 0.002, p = .962) , 9 letter (F (1,159) = 0.169, p = .682) or time x treatment interaction in 5 letter (F (1,159) = 0.261, p = .610), 7 letter (F (1,159) = 1.200, p = .274) , 9 letter (F (1,159) = 0.446, p = .505). In terms of the flanker interference effect, a repeated measures ANOVA revealed a significant group x time interaction. This effect on executive function was supported by Scholey et al (2010) found that young adults showed significantly improved performance on a serial three's subtraction task following an acute flavonoid intervention again indicating improved this performance.

When letter memory response times were combined with flanker interference effect to form a composite score for executive function, a significant main effect of time and time X group interaction were seen. This finding confirms the evidence that supplementation of fruit flavonoids can produce improvements in executive function. These findings also suggest that the domain of executive function may be particularly sensitive to fruit and flavonoids intervention.

To further probe this potential effect on executive function, in Chapter 4 three further executive function tasks were employed: letter memory, flanker task and StopGo. Unfortunately, none of these tasks showed any significant group or group X time differences. A main effect of time was found for letter memory response time, however when the control group was removed from the analysis, no significant differences between performance following HF compared to LF were seen. When measures from the executive function tasks were combined to produce a composite measure of executive function, no significant differences in time, group or time x group interactions were evident. It is clear from these data that the result from this Chapter do not replicate the results seen in Chapter 3. The lack of detection an effect of flavonoids on executive function in Chapter 4 may have been influenced by the reduced length of intervention, which was nearly half (7 weeks compared to 12 weeks). It was suggested that measurable improvement in this function might be shown with longer length of intervention. However, an assessment of the effect size seen in Chapter 3 with that in Chapter 4 leads to the possibility that there may have been a problem with the power of the sample in Chapter 4.

In an attempt to overcome any issues with power and sample size, a further study to follow-up the effect of flavonoids fruit on cognitive function (Chapter 5) used a much larger sample size than previously tested. Following supplementation with 80 grams portion of low- or high-flavonoid fruit, participants showed a main effect of time on letter memory response time although this was not supported by a main effect of either treatment or time X treatment interaction, suggesting this improvement reflected a practice effect. When considering composite scores of executive function, no association were seen between treatment and performance with only a significant effect of time on composite score of TMT, Stroop, and verbal fluency, which again can be attributed to the effects of practice. In a previous study by Mastroiacovo et al. (2015) they found a positive effect of TMT as a function of flavonoid intake. Verbal Fluency was also used in one study with a positive effect shown (Mastroiacovo et al., 2015). The current results failed to show differential benefits of flavonoids fruit intervention on cognitive performance, suggesting that 80 grams of flavonoids were either not adequate to improve cognitive function in the sample or the cognitive tasks used were not sensitive enough to detect the cognitive improvement from flavonoids. Overall, therefore, whilst the result from Chapter 3 found some evidence that executive function may improve as a function of 12 weeks rich flavonoids fruit supplementation when compared to low flavonoids, the findings from Chapter 4 and 5 failed to replicate these effects. Future studies will need to ensure that they employ suitably sensitive cognitive tasks in order detect any small effects of nutritional intervention. Alternatively, supplementation of only 40 – 80 grams of rich flavonoids fruit during 7-12 weeks may show a small effect in real world to improve executive function following nutrition intervention, which was more confounder effected.

6.2.3 Processing speed

Previous studies have shown that the DSST was sensitive to measure processing speed in isoflavones study (Casini, 2006) and a ginkgo biloba study (Santos et al., 2003; Stough, Clarke, Lloyd, & Nathan, 2001). However, the findings from Chapter 3, and again in Chapter 5, do not support the evidence that processing speed is affected by flavonoid intervention.

This is similar to Macready (2012) who investigated the effects of supplementation with flavonoids from fruits and vegetables on cognitive performance in adult participants. Here, she showed no post intervention between group differences on the DSST task. Similarly, no associations between flavonoids and DSST have been found in previous studies with flavonoid-rich orange juice (Alharbi et al., 2015; Kean et al., 2015). In both Chapter 3 and Chapter 5, repeated ANOVA analysis revealed the effect of time but no treatment, or time X treatment interaction.

It is possible that the processing speed task may not be sufficiently sensitive to evaluate the difference between flavonoids chronic interventions, or processing speed function itself may not be sensitive to flavonoid manipulation. Inconsistent finding of this task in previous studies using adult participants, the domain of processing speed has been found to demonstrate the effect of flavonoids but not in my studies, suggesting that the DSST is not sensitive enough to detect the improvement that may induce from the chronic consumption of flavonoids in 40 or 80 grams fruit. Further studies should consider using more other tasks to assess processing speed including finger tapping (Alharbi et al., 2015), RVIP (Scholey et al., 2010; A. W. Watson et al., 2015), and choice reaction time (Field et al., 2011).

6.2.4 Mood affect

The findings from Chapter 3 showed an inverse effect of flavonoid-rich fruit on negative affect with a 40 grams portion of high-flavonoid fruit for 12 weeks producing a decrease in negative mood score, whereas low-flavonoid fruit producing an increase in this score. Previous studies have also shown changes in mood following flavonoid interventions, particularly cocoa interventions (Massee et al., 2015; Scholey et al., 2010). In Chapter 4, I attempted to replicate the mood effects we had seen in Chapter 3. Unfortunately supplementation with 80 grams of flavonoid-rich fruit failed to replicate the reduction in negative mood, although an average raw score was seen showing that participants consuming the low flavonoid fruit had higher negative affect scores than participants consuming the flavonoid-rich fruit. Again, the failure to detect an effect of flavonoids on mood in Chapter 4 may have been influenced by the shorter length of supplementation (7 weeks compared to 12 weeks) or by power and sample size, that in Chapter 4 had only 44 HF and 43 LF participants remain in analysis after exclusion. In Chapter 5 the length of intervention was 12 weeks and a large number of participants were tested, but here no significant differences in positive and negative affect were seen. It should be noted that in both Chapter 3 and 4, the mood measure was taken shortly after the participants had completed their O-NET tests. However, in Chapter 5 I was unable to measure at the same time point (the O-NET was unexpectedly postponed by a few weeks) and therefore, mood was measured at the end of the intervention period approximately 1 month before the O-NET test. It is possible that the difference in timing resulted in the lack of replication of the reduction in negative mood. This may have explained by the students had more stress and pressure under the preparation of the test. It is also possible that the mood measure is not adequate to demonstrate subtle differences and changes in affect following chronic flavonoid fruit intervention or the mood may not influenced by consumption of flavonoids in fruit. However, a failure to detect the effect of flavonoids on mood was also observed by Kean et al. (2015) who assessed mood following 8 weeks of supplementation with a high-flavanone orange juice (containing 305 mg flavanone).

6.2.5 O-NET Score

In Thailand, the O-NET score is used to demonstrate academic achievement. As can be seen from the literature reviewed in Chapter 1, a number of studies have shown that children's diet in children is important for their cognitive development and performance, and is particularly influential on their academic achievement in school (Bellisle, 2004; Benton, 2010; Theodore et al., 2009). Specifically, there is evidence that supplementation with multiple micronutrients or even a single nutrient (such as folate and iodine) can improve academic outcome in children. However, no study to date has focussed on the relationship between flavonoid consumption and academic outcomes. This study is the first intervention trial to evaluate the effect of a flavonoid-rich fruit on academic attainment. Unfortunately, there is no evidence from any of my studies that long-term supplementation with a flavonoid-rich or –poor fruit can produce any difference in academic attainment, as no difference between the groups were found for their O-NET score. As with the cognitive domains summarised earlier, O-NET score was unaffected by the consumption of either 40 grams or 80 grams of low- or high-flavonoid fruit. Even though in Chapter 3 we found an improvement in the executive function composite score, this has no association with educational attainment.

Education attainment can be affected by multiple factors and it is, therefore, difficult to rule out any single influential factor. Nutrition certainly plays a major role in improving academic attainment, with evidence coming from several sources. Taras (2005) concluded that when considering nutrition and academic performance, students with anemia caused by iron deficiencies had poor academic outcome. Insufficiency of micronutrient such as zinc and iodine are also associated with academic disadvantages (Taras, 2005). Further serious problems affecting learning ability have been reported in children with nutrient insufficiencies

(Benton, 2010) and fasting (Benton, 2010; Simeon & Grantham-McGregor, 1989). In my studies, I have not screened for children with poor nutritional status which may have introduced an additional source of variance in the data. Perhaps more importantly, although not firmly established, it may be that deficiencies in nutritional status may be more important a determinant of performance than small and incremental increases in nutritional status above some baseline. Future work is needed to explore this.

6.3 Limitation of the studies

There are several limitations in the current thesis which need to be considered;

First of all, although this RCT study employed participants who were double-blind and comparable at baseline, we decided to use a between participant design. An alternative would have been to use a within subject design, which would have taken into account the different development rate in children that would have provided more sensitive measurement of the improvement from long term supplementation of rich flavonoid fruits. The reason for not using a within participant design was primarily a practical one given the need to work within the constraints of school semesters. Although, the result can be generalized to children with similar characteristic and socioeconomic status in northeast Thailand, which are children in municipality school with low and low-middle socioeconomic status. Caution is needed if generalized to other groups of children with difference characteristic.

Secondly, the dose of flavonoids used in this study was quite low in comparison to other studies. The LF group received 227.80 to 1152.81 µg flavonoids daily, whereas the HF group received 6245.15 to 13257.70 μg from the intervention. Unfortunately, it was not possible to measure the actual daily flavonoids intake per participant because the FFQ had limited choice of fruit, vegetable, juice, and soy products to be used in the calculation. Although the FFQ was developed carefully, it had some weaknesses to investigate food consumption, particularly in this age group and as a self- report measure, it is possible the participants over- or under-estimated their habitual consumption. In Chapter 3, the amount of fruit and vegetable consumption from FFQ in HF condition was higher than LF condition, which might show the positive effect on cognitive performance in this group. No significant different were found in consumption of fruit and vegetable between HF, LF or control condition in Chapter 4. However, in Chapter 5, the LF condition consumed more fruit and vegetable than HF condition. Related to the difference of fruit and vegetable consumption from FFQ, the participant in LF condition with particularly high level of consumption was excluded from the cognitive analysis. The analysis revealed no significant difference between LF and HF condition. In comparison to other studies, doses typically used in acute intervention range typically between 250 to 994 mg of cocoa flavonoid and about 6 portions of fruit and vegetable compared to my studies with 62-132 mg in HF and 2 -11 mg in LF. The different classes of flavonoids found in the different food used and have been found positive effect on cognitive performance such flavanols (chocolate) (Brickman et al., 2014; Mastroiacovo et al., 2015), flavone (orange juice)(Kean et al., 2015) and proanthocyanidins (Pipingas et al., 2008). Moreover, the studies that show positive effects are often RCTs that supplement with a single flavonoid or flavonoids extracts that do not comprise a whole food. In my studies, total flavonoids from whole fruits were supplemented, which is difference from previous studies that reached significant effect of a single flavonoid or flavonoids extracts. Only the study by Macready (2012) uses a similar design. Here, they supplemented 161 participants with 2 (LF), 4 (MF) and 6 (HF) portions of fruit and vegetables and saw an improvement in spatial working memory. No changes on executive function, processing speed and mood were observed. To date there is no evidence to confirm the exact amount of flavonoids required to optimize cognitive function. The failure to reliably show positive effects in this study may therefore result from inadequate flavonoid content. Alternatively, there was some evidence in the current studies that children consumed more fruit in the school whilst at the same time reducing intake at home. Cooperation with parents and carers, who were generally supportive of the scheme, may have led them to provide fewer fruit and vegetables at home, believing children had an adequate intake at school (Ransley et al., 2007). This may have limited the potential to show any benefit of the intervention. Furthermore, a 40 or 80 gram portion of fruit supplementation may not enough to demonstrate subtle difference and change in an academic environment in terms of cognitive performance and mood. This could be consider in two ways: 1) perhaps extending the length of the intervention over a period of years might produce measurable effects; and 2) there are of course lots of difference phytochemical compounds in fruit which may be having an effect in LF condition such as carotenoid and vitamin C.

Thirdly, cognitive tasks used in this study were identified through significant findings from previous flavonoid studies, which often focussed on related measures targeting cognitive domains. However, the majority of these studies were carried out in adult and older adult participants and may not be applicable for children. Of the studies reviewed, only the studies of Whyte et al. (2015) and Whyte and Williams (2015) were conducted with children, and they showed a positive effect of single flavonoid supplementation on the performance of an AVLT task. To ensure that task was suitable for Thai children, the AVLT Thai language version in current thesis was matched for concreteness and familiarity. Furthermore, Thai vocabulary from textbooks and handbooks of Thai subject of grade 6 student were used to create the task. It is possibly that tests used in previous flavonoid studies with adult participants may not be sensitive enough to detect the change of cognitive performance in children, or the degree of change in cognitive ability of children following flavonoid supplementation may be different from adults.

Fourthly, the unexpected postponement of the O-NET test in Chapter 5 caused there to be a 1 month gap between the end of intervention and academic measure. Previously, participants were measured on the cognitive tasks and mood scales during the pressure of the O-NET test, this was not possible during Chapter 5. Obviously, the lapse between the finish of the intervention and administration of the O-NET measure could have contributed to the null effect observed. The decay time course of flavonoids is poorly understood but a 1 month gap is often used as a wash out period in within participant designs. Finally, the O-NET measure had no baseline, and we assumed that there were no differences between groups as they were well matched. Possibly, there were small within participant changes as a function of the intervention that were not picked up given the absence of a baseline for the measure.

Fifthly, the intervention fruit was only offered to participant in school 5 days per week, not during the weekends. Importantly, the intervention period was carried out near the end of the academic year, which was very busy and contained a number of public holidays. Therefore, even though the length of intervention was 12 weeks (Chapter 3 and 5) or 7 weeks (Chapter 4), the fruit interventions were only administered for 53, 35, 52 days in Chapter 3, 4 and 5 respectively. It may be the inconsistency of supplementation that may have affected the data in this thesis as participants only received their intervention when they were in school.

Sixthly, the test environment also needs to be considered. All participants were tested in school and there were factors such as classroom and playground noise which may have impacted on the administration and completion of the cognitive tasks.

Finally, at baseline all grade 6 students were recruited to the study without screening their nutritional status, for any micro or macro nutrient deficiencies or any other potential prognostic factors that may have affected the results. As shown earlier, a number of studies have shown that cognitive performance and academic attainment, can be influence by these factors. One might also speculate that the positive effects of flavonoids would be most likely to be observed in those children with the poorest nutritional status at baseline.

In summary, there are several limitations of these studies that should be considered alongside the data obtained. The effects of flavonoids supplementation on cognitive performance related to school achievement was required to replicate for confirm and validate the results that may important for the school children stage.

6.4 Future directions

The positive effects of flavonoid fruit supplementation were found on cognitive performance and mood in children aged 11-13 years old. This is the first study in Thailand that expands the hypothesised of potential effects of flavonoids. This indicates two kind of studies which may be considered next in Thailand. To begin with a prospective study to identify the nutrition intake associated cognitive performance and academic attainment in children may provide the pilot evidence in Thailand to support the hypothesis of positive effects of flavonoid on cognitive development in children. Secondly, longer chronic interventions may help to provide evidence regarding the optimal dose or times required to observe effects of flavonoids.Williams et al. (2008) found that the improvement of cognitive performance following blueberry supplementation in animals occurred after 3 weeks and persisted during 12 weeks test period. The association between flavonoids consumption and cognitive performance in current studies were continuing to12 weeks and may have peaked with a longer duration of study.

To evaluate academic attainment in this thesis, a nationally available O-NET task was employed. There was no baseline of O-NET score to compare with post intervention due to the fact that this test consisted of the eight subjects in the core curriculum are learned from grade 1 to grade 6. Future research may consider using better pre and post measures to assess academic outcomes such as grade point average, and performance in individual subject (science, Thai, mathematics etc.).

Previous interventions have focused on supplementation with flavonoid extracts, compounds or capsules. As in the current thesis, other researchers have encouraged research that focuses on the role of flavonoids as part of whole foods as detecting the positive effect of flavonoids within the context of natural and habitual diet is obviously safe (Kay, 2010).

The intervention fruits used in current studies were based on the analysis of flavonoids contents from Kongkachuichai et al. (2010) who analysed carotenoid and flavonoid profiles as well as the dietary fibre of fruits commonly consumed in Thailand. The limited database of flavonoid in Thai fruit, which are different from other country (due to the difference of growing environment), this suggest that the research to support Thai databases of flavonoids have significant room to be progressed, which are reliable and valid for use in further flavonoids supplementation.

In terms of dose response to optimise the benefit of flavonoids, previous studies were administered as can be seen in previous RCT studies (Macready, 2012) of fruit and vegetable ≥ 6 portions per day, while in our studies we provided only 1 portion (40 grams) in Chapter 3 and 2 portions (80 grams) in Chapter 4 and 5. At face value this seems to be insufficient to produce effects on cognitive performance. However, more work is needed to determine what an appropriate dose might be, whereas the finding from FFQ in this study show 4-8 portions of fruit and vegetable intake in participants including the intervention fruit, the bias in terms of tendency to over report intake may be considered. Further studies should include better measures to assess the dietary intake with more accuracy.

The schools selected for the current studies were municipal schools located in urban areas and were selected on the basis for ease of running the intervention. This may have affected the results of studies that children in urban and rural have quite different baseline socioeconomic features and also eating behaviours, which affect their performance. Therefore, further studies should employ more systematic sampling procedures to control confounds and to generalize across different demographic populations.

The positive findings of executive function in Chapter 3 was shown by a computer base task, whereas in Chapter 5 failed to replicate the effect on this domain. One possible reason for the inconsistencies are the absence of data on the validity and sensitivity of the measures employed, which comprised both computer based and pencil and paper based cognitive tasks across studies. Further studies that use measures that have better validity and sensitivity characteristics will be important in helping the field to move forward.

6.5 Conclusion

In conclusion, the current thesis reports the first attempt to study the effects of chronic supplementation with flavonoid-rich and flavonoid-poor fruits on academic attainment, cognitive performance and mood effects in 11-13 year old Thai children. The findings showed some evidence that a composite score of executive function might be influenced by chronic intake of flavonoid rich fruits in comparison to control samples with low flavonoids fruits. It was also found that mood, particularly negative affect was shown interaction between time and groups effect following chronic intervention of flavonoids fruit base. Unfortunately, these findings were not replicated throughout the series of studies meaning that the initial result must be viewed with caution.

Overall the findings do not provide evidence that regular supplementation with flavonoids in children can support better academic outcomes and boost cognitive performance or mood. Further research should consider the appropriate flavonoid dose to elicit a response, as well as whether the cognitive tasks are sufficiently sensitive to detect a subtle effect of a nutritional intervention. It is clear, however, that there is huge potential for nutritional interventions in school aged populations to optimise academic performance.

References

Alharbi, M. H., Lamport, D. J., Dodd, G. F., Sunders, C., Harkness, L., Butler, L., & Spencer, J. P. (2015). Flavonoid-rich orange juice is associated with acute improvements in cognitive function in healthy middle-aed males. *European journal of nutrition*. doi: 10.1007/s00394-015-1016-9

Anderson, A. S., Porteous, L. E. G., Foster, E., Higgins, C., Stead, M., Hetherington, M., . . . Adamson, A. J. (2004). The impact of a school-based nutrition education intervention on dietary intake and cognitive and attitudinal variables relating to fruits and vegetables. *Public health nutrition, 8*(6), 650-656. doi: 10.1079/phn2004721

Anderson, A. S., T., F., Manly, T., & Robertson, I. (1998). Attentional skills following traumatic brain injury in childhood: a componential analysis. *Brain Injury, 12*(11), 937-949. doi: 10.1080/026990598121990

Armijo-Olivo, S., Warren, S., & Magee, D. (2009). Intention to treat analysis, compliance, drop-outs and how to deal with missing data in clinical research: a review. *Physical Therapy Reviews, 14*(1), 36-49. doi: 10.1179/174328809X405928

Baddeley, A. (1998). The central executive: a concept and some misconceptions. *Journal of the International Neuropsychological Society, 4*, 523-526.

Bellisle, F. (2004). Effects of diet on behaviour and cognition in children. *The British journal of nutrition, 92*(S2), S227-s232. doi: 10.1079/bjn20041171

Benton, D. (2010). The influence of dietary status on the cognitive performance of children. *Molecular nutrition & food research, 54*(4), 457-470. doi: 10.1002/mnfr.200900158

Bhagwat, S., Haytowitz, D. B., & Holden, J. M. (2013). USDA Database for the Flavonoid Content of Selected Foods.

Bondonno, C. P., Downey, L. A., Croft, K. D., Scholey, A., Stough, C., Yang, X., . . . Hodgson, J. M. (2014). The acute effect of flavonoid-rich apples and nitrate-rich spinach on cognitive performance and mood in healthy men and women. *Food & function, 5*(5), 849-858. doi: 10.1039/c3fo60590f

Brickman, A. M., Khan, U. A., Provenzano, F. A., Yeung, L. K., Suzuki, W., Schroeter, H., . . . Small, S. A. (2014). Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults. *Nature neuroscience, 17*(12), 1798-1803. doi: 10.1038/nn.3850

Bryan, J. (1998). Cognitive function and its links with nutrition. *Proc Nutr Soc, 22*, 211-215.

Butchart, C., Kyle, J., McNeill, G., Corley, J., Gow, A. J., Starr, J. M., & Deary, I. J. (2011). Flavonoid intake in relation to cognitive function in later life in the Lothian Birth Cohort 1936. *The British journal of nutrition, 106*(1), 141-148. doi: 10.1017/S0007114510005738

Calderón-Garcidueñas, L., Mora-Tiscareño, A., Maricela Franco-Lira, M., Cross, J. V., Engle, R., Aragón-Flores, M., . . . D'Angiulli, A. (2013). Flavonol-rich dark cocoa significantly decreases plasma endothelin-1 and improves cognition in urban children. *Frontiers in pharmacology, 4*. doi: 10.3389/fphar.2013.00104

Camfield, D. A., Scholey, A., Pipingas, A., Silberstein, R., Kras, M., Nolidin, K., . . . Stough, C. (2012). Steady state visually evoked potential (SSVEP) topography changes associated with cocoa flavanol consumption. *Physiology & Behavior, 105*(4), 948-957. doi: 10.1016/j.physbeh.2011.11.013

Casini, M., Marelli, G., Papaleo, E., Ferrari, A., D'Ambrosio, F., Unfer, V. (2006). Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebo-controlled study *Fertil Steril, 85*, 972-978.

Cassidy, A., Huang, T., Rice, M., Rimm, E., & Tworoger, S. (2014). Intake of dietary flavonoids and risk of epithelial ovarian cancer. *The American Journal of Clinical Nutrition, 100*(5), 1344-1351. doi: 10.3945/ajcn.114.088708

Chang, S.-C., Cassidy, A., Willett, W. C., Rimm, E. B., O'Reilly, E. J., & Okereke, O. I. (2016). Dietary flavonoid intake and risk of incident depression in midlife and older women. *The American Journal of Clinical Nutrition*. doi: 10.3945/ajcn.115.124545

Chaytor, N., Schmitter-Edgecombe, M., & Burr, R. (2006). Improving the ecological validity of executive functioning assessment. *Archives of Clinical Neuropsychology, 21*, 217-227.

Cohen, M. (1997). *The Children's Memory Scale*. San Antonio: Harcourt Brace & Company.

Commenges, D., Scotet, V., Renaud, S., Jacqmin-Gadda, H., Barberger-Gateau, P., & Dartigues, J.-F. (2000). Intake of ¯avonoids and risk of dementia. *European Journal of Epidemiology, 16*, 357-363.

Crews Jr, W. D., Harrison, D. W., & Wright, J. W. (2008a). A double-blind , placebo-controlled , randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health : clinical findings from a sample of healthy , cognitively intact older. *Am J Clin Nutr, 87*, 872-880.

Crews Jr, W. D., Harrison, D. W., & Wright, J. W. (2008b). A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. *The American Journal of Clinical Nutrition, 87*, 872-880.

Cutler, G., Nettleton, J., Ross, J., Harnack, L., Jacobs, D. J., Scrafford, C., . . . Robien, K. (2008). Dietary flavonoid intake and risk of cancer in postmenopausal women: the Iowa Women's Health Study. *International Journal of Cancer 123*(3), 664-671. doi: 10.1002/ijc.23564 de Jager, C. A., Dye, L., de Bruin, E. A., Butler, L., Fletcher, J., Lamport, D. J., . . . Wesnes, K. (2014). Criteria for validation and selection of cognitive tests for investigating the effects of foods and nutrients. *Nutrition reviews, 72*(3), 162-179. doi: 10.1111/nure.12094

Devore, E. E., Kang, J. H., Breteler, M. M., & Grodstein, F. (2012). Dietary intakes of berries and flavonoids in relation to cognitive decline. *Annals of neurology, 72*(1), 135-143. doi: 10.1002/ana.23594

Eilander, A., Gera, T., Sachdev, H. S., Transler, C., van der Knaap, H. C., Kok, F. J., & Osendarp, S. J. (2010). Multiple micronutrient supplementation for improving cognitive performance in children: systematic review of randomized controlled trials. *The American Journal of Clinical Nutrition, 91*(1), 115-130. doi: 10.3945/ajcn.2009.28376

Field, D. T., Williams, C. M., & Butler, L. (2011). Consumption of cocoa flavanols results in an acute improvement in visual and cognitive function. *Physiology & Behavior, 103*, 255-260. doi: 10.1016/j.physbeh.2011.02.013

Ford, P. A., Jaceldo-Siegl, K., Lee, J. W., Youngberg, W., & Tonstad, S. (2013). Intake of Mediterranean foods associated with positive affect and low negative affect. *Journal of psychosomatic research, 74*(2), 142-148. doi: 10.1016/j.jpsychores.2012.11.002

Francis, S. T., Head, K., Morris, P. G., & Macdonald, I. A. (2006). The Effect of Flavanol-rich Cocoa on the fMRI Response to a Cognitive Task in Healthy Young People. *Journal of Cardiovascular Pharmacology, 47*(Suppl 2), s215-220.

Garcia-Lafuente, A., Guillamon, E., Villares, A., Rostagno, M. A., & Martinez, J. A. (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm Res, 58*(9), 537-552. doi: 10.1007/s00011-009-0037-3

Gomar, A., Hosseini, A., & Mirazi, N. (2014). Preventive effect of Rubus fruticosus on learning and memory impairment in an experimental model of diabetic neuropathy in male rats. *PharmaNutrition, 2*(4), 155-160. doi: http://dx.doi.org/10.1016/j.phanu.2014.09.001

Haskell, C. F., Scholey, A. B., Jackson, P. A., Elliott, J. M., Defeyter, M. A., Greer, J., . . . Kennedy, D. O. (2008). Cognitive and mood effects in healthy children during 12 weeks' supplementation with multi-vitamin/minerals. *The British journal of nutrition, 100*(5), 1086-1096. doi: 10.1017/S0007114508959213

Homack, S., Lee, D., & Riccio, C. A. (2005). Test review: Delis-Kaplan executive function system. *Journal of clinical and experimental neuropsychology, 27*(5), 599-609. doi: 10.1080/13803390490918444

Hughes, D., & Bryan, J. (2003). The Assessment of Cognitive Performance in Children: Considerations for Detecting Nutritional Influences. *Nutrition Reviews, 61*(12), 413-422. doi: 10.1301/nr.2003.dec.413-422

Hui, C., Qi, X., Qianyong, Z., Xiaoli, P., Jundong, Z., & Mantian, M. (2013). Flavonoids, Flavonoid Subclasses and Breast Cancer Risk: A Meta-Analysis of Epidemiologic Studies. *PLOS ONE, 8*(1), e54318. doi: 10.1371/journal.pone.0054318

Jacka, F. N., Pasco, J. A., Mykletun, A., Williams, L. J., Hodge, A. M., O'Reilly, S. L., . . . Berk, M. (2010). Association of Western and Traditional Diets With Depression and Anxiety in Women. *American Journal of Psychiatry, 167*(3), 305-311. doi: 10.1176/appi.ajp.2009.09060881

Jacques, P. F., Cassidy, A., Rogers, G., Peterson, J. J., & Dwyer, J. T. (2015). Dietary flavonoid intakes and CVD incidence in the Framingham Offspring Cohort. *The British journal of nutrition, 114*(9), 1496-1503. doi: 10.1017/S0007114515003141

Kail, R. (1991). Developmental Change in Speed of Processing During Childhood and Adolescence *Psychological Bulletin, 109*, 490-501.

Kail, R. (2000). Speed of information processing: developmental

change and links to intelligence. . *J Sch Psychol., 38*, 51-61.

Kang, J. H., Ascherio, A., & Grodstein, F. (2005). Fruit and vegetable consumption and cognitive decline in aging women. *Annals of neurology, 57*(5), 713-720. doi: 10.1002/ana.20476

Kay, C. D. (2010). The future of flavonoid research. *The British journal of nutrition, 104 Suppl 3*, S91-95. doi: 10.1017/S000711451000396X

Kean, R. J., Lamport, D. J., Dodd, G. F., Freeman, J. E., Williams, C. M., Ellis, J. A., . . . Spencer, J. P. (2015). Chronic consumption of flavanone-rich orange juice is associated with cognitive benefits: an 8-wk, randomized, double-blind, placebo-controlled trial in healthy older adults. *The American Journal of Clinical Nutrition, 101*(3), 506-514. doi: 10.3945/ajcn.114.088518

Keck, A.-S., Qiao, Q., & Jeffery, E. H. (2003). Food Matrix Effects on Bioactivity of Broccoli-Derived Sulforaphane in Liver and Colon of F344 Rats. *Journal of Agricultural and Food Chemistry, 51*(11), 3320-3327. doi: 10.1021/jf026189a

Kent, K., Charlton, K., Roodenrys, S., Batterham, M., Potter, J., Traynor, V., . . . Richards, R. (2015). Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. *European Journal of Nutrition*, 1-9. doi: 10.1007/s00394-015-1083-y

Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., . . . Aromaa, A. (2002). Flavonoid intake and risk of chronic diseases. *The American Journal of Clinical Nutrition, 76*(3), 560-568.

Kongkachuichai, R., Charoensiri, R., & Sungpuag, P. (2010). Carotenoid, flavonoid profiles and dietary fiber contents of fruits commonly consumed in Thailand. *International journal of food sciences and nutrition, 61*(5), 536-548. doi: 10.3109/09637481003677308

Lamport, D. J., Pal, D., Macready, A. L., Boucas, S. B., Fletcher, J. M., Williams, C. M., . . . Butler, L. T. (2015). The effects of flavanone-rich citrus juice on cognitive function and cerebral blood flow: An acute, randomised, placebo controlled crossover trial in healthy young adults. . *Psychopharmacology submitted for publication*.

Lamport, D. J., Pal, D., Moutsiana, C., Field, D. T., Williams, C. M., Spencer, J. P., & Butler, L. T. (2015). The effect of flavanol-rich cocoa on cerebral perfusion in healthy older adults during conscious resting state: a placebo controlled, crossover, acute trial. *Psychopharmacology, 232*(17), 3227-3234. doi: 10.1007/s00213-015-3972-4

Lee, J., Pase, M., Pipingas, A., J., R., Thurgood, M., Villalon, L., . . . Scholey, A. (2015). Switching to a 10-day Mediterranean-style diet improves mood and cardiovascular function in a controlled crossover study. *Nutrition, 31*(5), 647-652. doi: 10.1016/j.nut.2014.10.008

Lee, K., Chen, Y., Lin, C., Chiu, W., Liao, H., & Lin, S. (2013). Consumption of Oriental Plums Improved the Cognitive Performance and Modulated the Cerebral Neurodegeneration-Related Protein Expressions in Rats with Nicotinamide/Streptozotocin-Induced Diabetes. *Food and Nutrition Sciences, 4*, 1145-1154. doi: 10.4236/fns.2013.411148

Leh, S. E., Petrides, M., & Strafella, A. P. (2010). The neural circuitry of executive functions in healthy subjects and Parkinson's disease. *Neuropsychopharmacology, 35*(1), 70-85. doi: 10.1038/npp.2009.88

Letenneur, L., Proust-Lima, C., Le Gouge, A., Dartigues, J. F., & Barberger-Gateau, P. (2007). Flavonoid intake and cognitive decline over a 10-year period. *American Journal of Epidemiology 165*(12), 1364-1371. doi: 10.1093/aje/kwm036

Lezak, M. (1995). *Neuropsychological Assessment*. New York: Oxford University Press.

Loef, M., & Walach, H. (2012). Fruit, vegetables and prevention of cognitive decline or dementia: a systematic review of cohort studies. *The journal of nutrition, health & aging, 16*(7), 626-630.

Macready, A. L. (2012). *The FLAVURS trial: The infuence of a flavonoid-rich versus flavonoid-poor fruit and vegetable dietary intervention on cognitive function in free-living individual at risk of cardiovascular disease.* (Ph.D.), Reading.

Macready, A. L., George, T. W., Chong, M. F., Alimbetov, D. S., Jin, Y., Vidal, A., . . . Group, F. S. (2014). Flavonoid-rich fruit and vegetables improve microvascular reactivity and inflammatory status in men at risk of cardiovascular disease--FLAVURS: a randomized controlled trial. *The American Journal of Clinical Nutrition, 99*(3), 479-489. doi: 10.3945/ajcn.113.074237

Maisuthisakul, P., Suttajit, M., & Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry, 100*(4), 1409-1418. doi: 10.1016/j.foodchem.2005.11.032

Mandel, S., Weinreb, O., Tamar Amit, & Youdim, M. B. H. (2004). Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-

gallate: implications for neurodegenerative diseases. *Journal of Neuroschemistry, 88*, 1555-1569. doi: 10.1046/j.1471-4159.2003.02291.x

Massee, L. A., Ried, K., Pase, M., Travica, N., Yoganathan, J., Scholey, A., . . . Pipingas, A. (2015). The acute and sub-chronic effects of cocoa flavanols on mood, cognitive and cardiovascular health in young healthy adults: a randomized, controlled trial. *Frontiers in pharmacology, 6*, 93. doi: 10.3389/fphar.2015.00093

Mastroiacovo, D., Kwik-Uribe, C., Grassi, D., Necozione, S., Raffaele, A., Pistacchio, L., . . . Desideri, G. (2015). Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study--a randomized controlled trial. *The American Journal of Clinical Nutrition, 101*(3), 538-548. doi: 10.3945/ajcn.114.092189

McCullough, M. L., Peterson, J. J., Patel, R., Jacques, P. F., Shah, R., & Dwyer, J. T. (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *The American Journal of Clinical Nutrition, 95*(2), 454-464. doi: 10.3945/ajcn.111.016634

McMartin, S. E., Jacka, F. N., & Colman, I. (2013). The association between fruit and vegetable consumption and mental health disorders: evidence from five waves of a national survey of Canadians. *Preventive medicine, 56*(3-4), 225-230. doi: 10.1016/j.ypmed.2012.12.016

McMillan, L., Owen, L., Kras, M., & Scholey, A. (2011). Behavioural effects of a 10-day Mediterranean diet. Results from a pilot study evaluating mood and cognitive performance. *Appetite, 56*, 143-147. doi: 10.1016/j.appet.2010.11.149

McMillan, L., Owen, L., Kras, M., & Scholey, A. (2011). Behavioural effects of a 10-day Mediterranean diet. Results from a pilot study evaluating mood and cognitive performance. *Appetite, 56*(1), 143-147. doi: http://dx.doi.org.idpproxy.reading.ac.uk/10.1016/j.appet.2010.11.149

Mennen, L. I., Walker, R., Bennetau-Pelissero, C., & Scalbert, A. (2005). Risks and safety of polyphenol consumption. *Am J Clin Nutr, 81*(1 Suppl), 326s-329s.

Ministry of Public Health (MOPH), T. (1995). The Fourth National Food and Nutrition Survey in Thailand, 1995. Nutrition Division,

Ministry of Public Health, Bangkok, Thailand. (inThai).

Ministry of Public Health, T. (2006). The Third National Health examination survey, 2003-2004. (in Thai): Health System Research Institute and Strategic and Planning

Office. Ministry of Public Health,Bangkok, Thailand.

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., & Wilson, R. S. (2006). Associations of vegetable and fruit consumption with age-related cognitive change. *Neurology, 67*(8), 1370-1376. doi: 10.1212/01.wnl.0000240224.38978.d8

Nimptsch, K., Zhang, X., Cassidy, A., Song, M., O'Reilly, E. J., Lin, J. H., . . . Wu, K. (2016). Habitual intake of flavonoid subclasses and risk of colorectal cancer in 2 large prospective cohorts. *The American Journal of Clinical Nutrition, 103*(1), 184-191. doi: 10.3945/ajcn.115.117507

Niu, K., Hozawa, A., Kuriyama, S., Ebihara, S., Guo, H., Nakaya, N., . . . Tsuji, I. (2009). Green tea consumption is associated with depressive symptoms in the elderly. *The American Journal of Clinical Nutrition, 90*, 1615-1622.

Nooyens, A. C., Bueno-de-Mesquita, H. B., van Boxtel, M. P., van Gelder, B. M., Verhagen, H., & Verschuren, W. M. (2011). Fruit and vegetable intake and cognitive decline in middle-aged men and women: the Doetinchem Cohort Study. *The British journal of nutrition, 106*(5), 752-761. doi: 10.1017/S0007114511001024

Nurk, E., Refsum, H., Drevon, C. A., Tell, G. S., Nygaard, H. A., Engedal, K., & Smith, A. D. (2009). Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *The Journal of nutrition, 139*(1), 120-127. doi: 10.3945/jn.108.095182

Nurk, E., Refsum, H., Drevon, C. A., Tell, G. S., Nygaard, H. A., Engedal, K., & Smith, A. D. (2010). Cognitive performance among the elderly in relation to the intake of plant foods. The Hordaland Health Study. *The British journal of nutrition, 104*(8), 1190-1201. doi: 10.1017/S0007114510001807

O'Neil, A., Quirk, S. E., Housden, S., Brennan, S. L., Williams, L. J., Pasco, J. A., . . . Jacka, F. N. (2014). Relationship Between Diet and Mental Health in Children and Adolescents: A Systematic Review. *American Journal of Public Health, 104*(10), e31-e42. doi: 10.2105/AJPH.2014.302110

Pastor-Valero, M., Furlan-Viebig, R., Menezes, P. R., da Silva, S. A., Vallada, H., & Scazufca, M. (2014). Education and WHO recommendations for fruit and vegetable intake are associated with better cognitive function in a disadvantaged Brazilian elderly population: a population-based cross-sectional study. *PLoS One, 9*(4), e94042. doi: 10.1371/journal.pone.0094042

Peltzer, K., & Pengpid, S. (2012). Fruits and vegetables consumption and associated factors among in-school adolescents in five Southeast Asian countries. *International journal of environmental research and public health, 9*(10), 3575-3587. doi: 10.3390/ijerph9103575

Peneau, S., Galan, P., Jeandel, C., Ferry, M., Andreeva, V., Hercberg, S., . . . Group, S. V. M. R. (2011). Fruit and vegetable intake and cognitive function in the SU.VI.MAX 2 prospective study. *The American Journal of Clinical Nutrition, 94*(5), 1295-1303. doi: 10.3945/ajcn.111.014712

Pipingas, A., Silberstein, R. B., Vitetta, L., Van Rooy, C., Harris, E. V., Young, J. M., . . . Nastasi, J. (2008). Improved Cognitive Performance after Dietary Supplementation with a Pinus radiata Bark Extract Formulation. *Phytotherapy Research* doi: 10.1002/ptr.2388

Polidori, M. C., Pratico, D., Mangialasche, F., Mariani, E., Aust, O., Anlasik, T., . . . Nelles, G. (2009). High fruit and vegetable intake is positively correlated with antioxidant status and cognitive performance in healthy subjects. *Journal of Alzheimer's disease, 17*(4), 921-927. doi: 10.3233/JAD-2009-1114

Pradhan, B., & Nagendra, H. R. (2009). Normative data for the digit-letter sustitution task in school chikdren. *International Journal of Yoga 2*(2), 69-72. doi: 10.4103/0973-6131.60047

Ransley, J. K., Greenwood, D. C., Cade, J. E., Blenkinsop, S., Schagen, I., Teeman, D., . . . Schagen, S. (2007). Does the school fruit and vegetable scheme improve children's diet? A non‐randomised controlled trial. *Journal of Epidemiology and Community Health, 61*(8), 699-703. doi: 10.1136/jech.2006.052696

Rendeiro, C., Foley, A., Lau, V. C., Ring, R., Rodriguez-Mateos, A., Vauzour, D., . . . Spencer, J. P. E. (2014). A role for hippocampal PSA-NCAM and NMDA-NR2B receptor function in flavonoid-induced spatial memory improvements in young rats. *Neuropharmacology, 79*(100), 335-344. doi: 10.1016/j.neuropharm.2013.12.003

Rendeiro, C., Vauzour, D., Rattray, M., Waffo-Teguo, P., Merillon, J. M., Butler, L. T., . . . Spencer, J. P. (2013). Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor. *PLoS One, 8*(5), e63535. doi: 10.1371/journal.pone.0063535

Richard, A., Rohrmann, S., Vandeleur, C. L., Mohler-Kuo, M., & Eichholzer, M. (2015). Associations between fruit and vegetable consumption and psychological distress: results from a population-based study. *BMC Psychiatry, 15*, 213. doi: 10.1186/s12888-015-0597-4

Rodriguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L., . . . Crozier, A. (2014). Bioavailability, bioactivity and impacts on health of dietary flavonoids and related compounds: an update. *Archives of Toxicology, 88*, 1803-1853. doi: 10.1007/s00204-014-13307

Romagnolo, D., & Selmin, O. (2012). Flavonoids and cancer prevention: a review of the evidence. *Journal of Nutrition in Gerontology and Geriatrics, 31*(3). doi: 10.1080/21551197.2012.702534

Santos, R. F., Galduroz, J. C., Barbieri, A., Castiglioni, M. L., Ytaya, L. Y., & Bueno, O. F. (2003). Cognitive performance, SPECT, and blood viscosity in elderly non-demented people using Ginkgo biloba. *Pharmacopsychiatry 36*, 127-133.

Satheannoppakao, W., Aekplakorn, W., & Pradipasen, M. (2009). Fruit and vegetable consumption and its recommended intake associated with sociodemographic factors: Thailand National Health Examination Survey III. *Public health nutrition, 12*(11), 2192-2198. doi: 10.1017/S1368980009005837

Scarmeas, N., Stern, Y., Tang, M., Mayeux, R., & Luchsinger, J. A. (2006). Mediterranean Diet and Risk for Alzheimer’s Disease. *Annals of neurology, 59*, 912-921.

Scholey, A. B., French, S. J., Morris, P. J., Kennedy, D. O., Milne, A. L., & Haskell, C. F. (2010). Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. *Journal of psychopharmacology, 24*(10), 1505-1514. doi: 10.1177/0269881109106923

Shukitt-Hale, B., Carey, A. N., Jenkins, D., Rabin, B. M., & Joseph, J. A. (2007). Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging. *Neurobiology of aging, 28*(8), 1187-1194. doi: 10.1016/j.neurobiolaging.2006.05.031

Shukitt-Hale, B., Carey, A. N., Simon, L., Mark, D. A., & Joseph, J. A. (2006). Effects of Concord grape juice on cognitive and motor deficits in aging. *Nutrition, 22*, 295-302. doi: 10.1016/j.nut.2005.07.016

Simeon, D. T., & Grantham-McGregor, S. (1989). Effects of missing breakfast on the cognitive functions of school children of differing nutritional status. *The American Journal of Clinical Nutrition, 49*, 646-653.

Spencer-Smith, M., & Anderson, V. (2009). Healthy and abnormal development of the prefrontal cortex. *Developmental Neurorehabilitation, 12*(5), 279-297. doi: 10.1080/17518420903090701

Spencer, J. P., Vafeiadou, K., Williams, R. J., & Vauzour, D. (2012). Neuroinflammation: modulation by flavonoids and mechanisms of action. *Molecular aspects of medicine, 33*(1), 83-97. doi: 10.1016/j.mam.2011.10.016

Stough, C., Clarke, J., Lloyd, J., & Nathan, P. J. (2001). Neuropsychological changes after 30-day Ginkgo biloba administration in healthy participants. *International Journal of Neuropsychopharmacology, 4*, 131-134.

Taras, H. (2005). Nutrition and Student Performance at School. *Journal of School Health, 75*(6), 199-213.

Thangthaeng, N., Poulose, S. M., Gomes, S. M., Miller, M. G., Bielinski, D. F., & Shukitt-Hale, B. (2016). Tart cherry supplementation improves working memory, hippocampal inflammation, and autophagy in aged rats. *AGE*, 1-12. doi: 10.1007/s11357-016-9945-7

Theodore, R. F., Thompson, J. M. D., Waldie, K. E., Wall, C., Becroft, D. M. O., Robinson, E., . . . Mitchell, E. A. (2009). Dietary patterns and intelligence in early and middle childhood. *Intelligence, 37*(5), 506-513. doi: 10.1016/j.intell.2009.07.001

Thilakarathna, S. H., & Rupasinghe, H. P. V. (2013). Flavonoid Bioavailability and Attempts for Bioavailability Enhancement. *Nutrients, 5*, 3367-3387. doi: 10.3390/nu5093367

Van der Elst, W., Dekker, S., Hurks, P., & Jolles, J. (2012). The Letter Digit Sustitution Test: Demograohic Influences ans Regression-Besed Normative Data for School-Aged Children. *Archives of Clinical Neuropsychology, 27*, 433-439. doi: 10.1093/arclin/acs045

Vazir, S., Nagalla, B., Thangiah, V., Kamasamudram, V., & Bhattiprolu, S. (2006). Effect of micronutrient supplement on health and nutritional status of schoolchildren: mental function. *Nutrition, 22*(1 Suppl), S26-32. doi: 10.1016/j.nut.2004.07.021

Wang, X., Ouyang, Y. Y., Liu, J., & Zhao, G. (2014). Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. *The British journal of nutrition, 111*(1), 1-11. doi: 10.1017/S000711451300278X

Watson, A. W., Haskell-Ramsay, C. F., Kennedy, D. O., Cooney, J. M., Trower, T., & Scheepens, A. (2015). Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults. *Journal of Functional Foods, 17*, 524-539. doi: 10.1016/j.jff.2015.06.005

Watson, D., & Clark, L. A. (1988). Development and Validation of Brief Measures of Positive and Negative Affect : The PANAS Scales. *Journal of Personality and Social Psychology, 54*(6), 1063-1070.

Whyte, A. R., Schafer, G., & Williams, C. M. (2015). Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. *European journal of nutrition*. doi: 10.1007/s00394-015-1029-4

Whyte, A. R., & Williams, C. M. (2015). Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10 y old children. *Nutrition, 31*(3), 531-534. doi: 10.1016/j.nut.2014.09.013

Williams, C. M., El Mohsen, M. A., Vauzour, D., Rendeiro, C., Butler, L. T., Ellis, J. A., . . . Spencer, J. P. (2008). Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radic Biol Med, 45*(3), 295-305. doi: 10.1016/j.freeradbiomed.2008.04.008

Working group on food-based dietary guidelines for Thai people: quantitative process. Institute of Nutrition, M. U. (2001). Thailand nutrition flag, Healthy eating for Thais.

Ye, X., Bhupathiraju, S. N., & Tucker, K. L. (2013). Variety in fruit and vegetable intake and cognitive function in middle-aged and older Puerto Rican adults. *The British journal of nutrition, 109*(3), 503-510. doi: 10.1017/S0007114512001183

Yuan, L., Ma, W., Liu, J., Meng, L., Liu, J., Li, S., . . . Xiao, R. (2014). Effects of GSTM1/GSTT1 gene polymorphism and fruit & vegetable consumption on antioxidant biomarkers and cognitive function in the elderly: a community based cross-sectional study. *PLoS One, 9*(11), e113588. doi: 10.1371/journal.pone.0113588

List of Appendices

Appendix A Ethical Approval

University of Reading

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Dr Laurie Butler
School of Psychology and Clinical Language Sciences
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9 September 2013

Dear Laurie

Research Ethics Committee Project 13/44: Effects of flavonoid-rich fruits on cognitive performance in Thai primary school students.
Provisional opinion

Thank you for submitting your proposal for this project, which has been considered by the Research Ethics Committee. Before the Committee allows the project to proceed, it asks that you address the following.

1. Re. Appendix A 'Participant Information Sheet' and Appendix B 'Consent Form'. The Committee asked that both of these documents be revised to use language more appropriate for children aged 11-12. The comment applies generally to these documents, but examples noted by the Committee include "*…and measure of other individual cognitive domains…*", "*A demographic questionnaire will be handed out in conjunction with a personal data sheet…*" in the PIS and "*…without detriment to any care or services I may be receiving…*" and "*…and eventual disposal of any identifiable material have been made clear to me…*" in the consent form. It was not, of course, possible for the Committee to tell if the Thai translations were in more accessible language. The Committee also suggested that consideration might be given to using separate Information Sheets and Consent Forms for parents and children, this being a very common 'best practice' when young people are involved as research subjects.
2. Re. Section 2.2 'Procedure'. The Committee asked for reassurance that the proposed tests, designed to assess cognitive performance were, a) validated and b) relevant to/appropriate for Thai schoolchildren of this age. The Committee asked to see copies of these tests (English language versions).
3. Re. Section 2.5 'Ethical Issues'. The Committee asked for reassurance that any local Thai requirements – comparable with the UK Disclosure and Barring Service (DBS, formerly CRB) checks would be made before the research commenced.
4. The Committee presumed that participants would be required to refrain from eating certain foodstuffs for a prescribed period to avoid interference with the effects of the study interventions. It asked that such restrictions, if required, be described in the procedures and in the participant information sheet.

In addition, one of the Committee's members with expertise in nutritional studies commented that the Daily Food Record sheets were confusing in places where, for example, it was not clear whether '1' (for plate of rice) or '3' (for serving spoons) should be entered in the portions column. No ethical concerns were raised, but it was suggested that revision might improve the chances of capturing accurate data.

I look forward to receiving a response from you, including updated documents where appropriate. Please use track changes, or other marking system, to highlight where changes have been made.

Yours sincerely

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Dr Claire Williams; Thitikan Ekathat

Appendix B Ethical Approval

University of Reading

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Dr Laurie Butler
School of Psychology and Clinical Language Sciences
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24 October 2014

Dear Laurie

UREC 13/44: Effects of flavonoid-rich fruits on cognitive performance in Thai primary school students. *Amendment favourable opinion*

Thank you for the request (email dated 9 October 2015, from Thitikan Ekathat and including attachment, refers) for amendment to UREC 13/44 (*extension until February 2016, increase in the number of participants, inclusion of a new school site, substitution of hard copy executive function tasks for computer-based versions*). I can confirm that the UREC Chair is happy for the project to continue on this basis.

Mike Proven

Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)
cc: Dr John Wright (Chair); Dr Claire Williams; Thitikan Ekathat

Appendix C PANAS Questionnaire (Thai)

ครั้งที่ 1 เลขที่............ห้อง............

แบบสอบเกี่ยวกับอารมณ์ (PANAS Questionnaire)

แบบสอบถามต่อไปนี้ประกอบไปด้วยคำที่อธิบายความรู้สึก และอารมณ์ต่างๆ หลังจากท่านอ่านแล้ว ให้ใส่ตัวเลขเพื่อบ่งบอกความรู้สึกของท่านว่ามีความรู้สึกหรืออารมณ์ต่างๆ ดังกล่าว เกิดขึ้นภายในสัปดาห์

1	**2**	**3**	**4**	**5**
เล็กน้อย / ไม่มี	เล็กน้อย	ปานกลาง	มาก	มากที่สุด

1. สนใจสิ่งต่างๆ	11. โกรธง่าย / ขี้โมโห
2. วิตกกังวล / ไม่มีความสุข	12. กระปรี้กระเปร่า
3. ตื่นเต้น / ดีใจ	13. ละอายใจ / อับอาย
4. เศร้า	14. มีแรงบันดาลใจ
5. มีพลัง เข้มแข็ง	15. ประหม่า
6. รู้สึกผิด	16. มุ่งมั่น / มั่นใจ
7. หวาดกลัว / กลัว	17. มีสมาธิ / ใส่ใจ
8. รู้สึกถูกคุกคาม	18. กระวนกระวายใจ
9. กระตือรือร้น	19. กระฉับกระเฉง
10. ภาคภูมิใจในตัวเอง	20. หวาดหวั่น / เกรงกลัว

Appendix C PANAS Questionnaire

Worksheet 3.1 The Positive and Negative Affect Schedule (PANAS; Watson et al., 1988)

PANAS Questionnaire

This scale consists of a number of words that describe different feelings and emotions. Read each item and then list the number from the scale below next to each word. **Indicate to what extent you feel this way right now, that is, at the present moment *OR* indicate the extent you have felt this way over the past week (circle the instructions you followed when taking this measure)**

1	2	3	4	5
Very Slightly or Not at All	A Little	Moderately	Quite a Bit	Extremely

__________ 1. Interested	__________ 11. Irritable
__________ 2. Distressed	__________ 12. Alert
__________ 3. Excited	__________ 13. Ashamed
__________ 4. Upset	__________ 14. Inspired
__________ 5. Strong	__________ 15. Nervous
__________ 6. Guilty	__________ 16. Determined
__________ 7. Scared	__________ 17. Attentive
__________ 8. Hostile	__________ 18. Jittery
__________ 9. Enthusiastic	__________ 19. Active
__________ 10. Proud	__________ 20. Afraid

Scoring Instructions:

Positive Affect Score: Add the scores on items 1, 3, 5, 9, 10, 12, 14, 16, 17, and 19. Scores can range from 10 – 50, with higher scores representing higher levels of positive affect. Mean Scores: Momentary = 29.7 (*SD* = 7.9); Weekly = 33.3 (*SD* = 7.2)

Negative Affect Score: Add the scores on items 2, 4, 6, 7, 8, 11, 13, 15, 18, and 20. Scores can range from 10 – 50, with lower scores representing lower levels of negative affect. Mean Score: Momentary = 14.8 (*SD* = 5.4); Weekly = 17.4 (*SD* = 6.2)

Appendix D FFQ

THE SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE FOR PRIMARY SCHOOL STUDENT OF WAT-GLANG MANICIPALITY SCHOOL, KHONKAEN PROVINCE, THAILAND

The Questionnaire is divided into 2 parts

Part 1 Personal information (8 items)

Part 2 Semi-Quantitative Food Frequency Questionnaire (23 items)

Past 1 PERSONAL INFORMATION

Please read each question and writing answers in the space provided, and writing "✔" in the brackets.

1. Student number………………… Room number………………………
2. I am now………………years old
3. Gender : ( ) Male ( ) Female
4. How many people in your household (including yourself)?.........................peoples.
5. What is your total household income per month?
   - ( ) 0 – 5,000 Bath
   - ( ) 5,001 – 10,000 Baht
   - ( ) 10,001 – 15,000 Baht
   - ( ) 15,001 – 20,000 Baht
   - ( ) 20,001 – 25,000 Baht
   - ( ) 25,001 – 30,000 Baht
   - ( ) More than 30,001 Baht
6. Weight……………………..Kg.
7. Height……………………...Cm.
8. Have you ever taken functional food/ Mineral for improving your health / brain?
   - ( ) No
   - ( ) Yes Identify………………..

Past 2 SEMI-QUANTITATIVE FOOD FREQUENCY QUESTIONAIRE

Listed below are food items that usually consumed. Please writing the number that reflect the amount of food you eaten and " ✔ " in the appropriate column to indicate how often, on average, you have eaten the amount of each food during the last year.

For example: If you eat stream rice 3 plate a day, you should put " ✔ " in the column Daily and write 9 portions in portion column.

Food items	Portion	Frequency							
		Never	Once per	2 per week	3 per week	4 per week	5 per	6 per	Daily
1. Rice Steam/sticky	9								✔

Carbohydrate Food Groups.

1 plate of rice = 3 rice-serving spoons = 3 Portion

1 bowl of noodles = 3 rice-serving spoons = 3 Portion

1 piece of fermented noodle = 1 rice-serving spoons = 1 Portion

1 slice of bread = 1 rice-serving spoons = 1 Portion

Food items	**Portion**	**Frequency**							
		Never	**Once per**	**2 per week**	**3 per week**	**4 per week**	**5 per**	**6 per**	**Daily**
1. Rice Steam/sticky									
2. Noodle / fermented noodle									
3. Bread/cake/biscuits									

Meat and Protein Food Groups.

1 egg = 2 spoons = 2 Portion

1 mackerel fish = 2 spoons = 2 Portion

1 chicken drumsticks = 2 spoons = 2 Portion

1 tofu = 4 spoons = 4 Portion

Yogurt = 1 cup = 1 Portion

Milk / soy milk 1 glass/ 1box = 1 Portion

Food items	**Portion**	**Frequency**							
		Never	**Once per week**	**2 per week**	**3 per week**	**4 per week**	**5 per week**	**6 per week**	**Daily**
4. Meat									
5. Fish									
6. Chicken / Duck									
7. other meat bacon/ham/meat ball/saugsage/nugget									
8. Milk									
9. Yogurt									
10. Soy milk									
11. Tofu									
12. Egg									
13. Nut / Bean									

Fruit and vegetable Food Groups.

1 portion of green vegetable = 1 rice-serving spoons (1/2 rice-serving spoons for cooked)

1 small banana/guava = 1 portion

1 orange = 1 portion

1 pieces of watermelon = 1 portion

6 little pieces of pineapple / papaya = 1 portion

Food items	**Portion**	**Frequency**							
		Never	**Once per week**	**2 per week**	**3 per week**	**4 per week**	**5 per week**	**6 per week**	**Daily**
14. Uncooked Green vegetable									
15. Cooked Green vegetable									
16. Other vegetable									
17. Fruit									
Pomelo									
Pineapple									
Guava									
Banana (Hom)									
Other fruit identify………									
18. Dry fruits									

Juice and snack Food Groups.

1 glass of juice / soft drink = 1 Portion

1 bowl of dessert = 1 Portion

1 bag of snack = 1 Portion

Food items	**Portion**	**Frequency**							
		Never	**Once per week**	**2 per week**	**3 per week**	**4 per week**	**5 per**	**6 per**	**Daily**
19. Fruit juice									
20. Snack									
21. Dessert									
22. Soft drink									
23. Candy / Sweet									

Appendix E Daily record

Daily food record Week……………………

Are you eating the following school lunch meal this week? Y/N

Please " ✔ " The appropriate column that reflects your eating for every row.

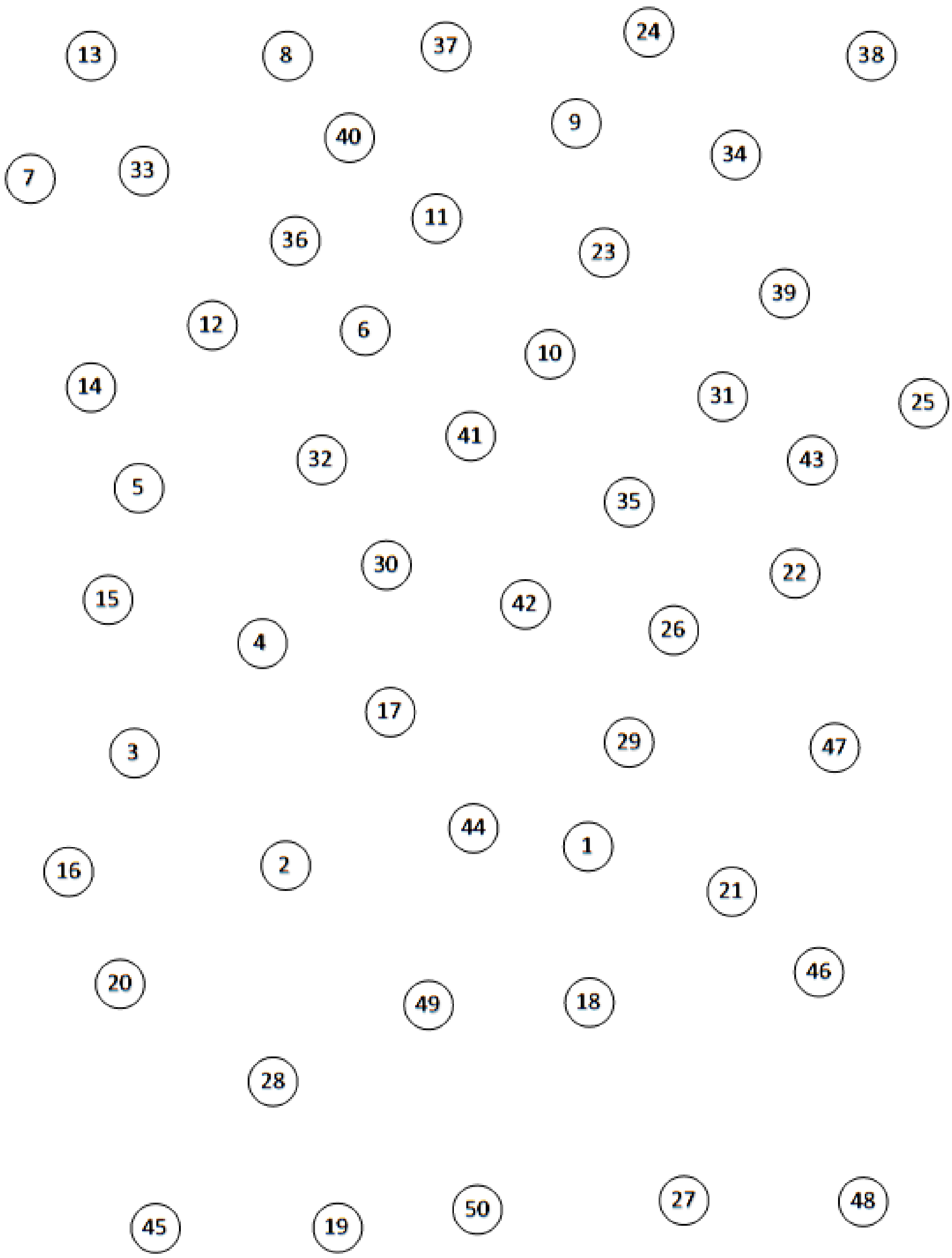
For example:

Day	Item	Consumed in total	Partially eaten	Not eaten at all
Monday	Main dish	✔		
	Fruits		✔	

Day	Item	Consumed in total	Partially eaten	Not eaten at all
Monday	Main dish			
	Fruits			
Tuesday	Main dish			
	Fruits			
Wednesday	Main dish			
	Fruits			
Thursday	Main dish			
	Fruits			
Friday	Main dish			
	Fruits			

Appendix F TMT task

ห้อง.................เลขที่...............



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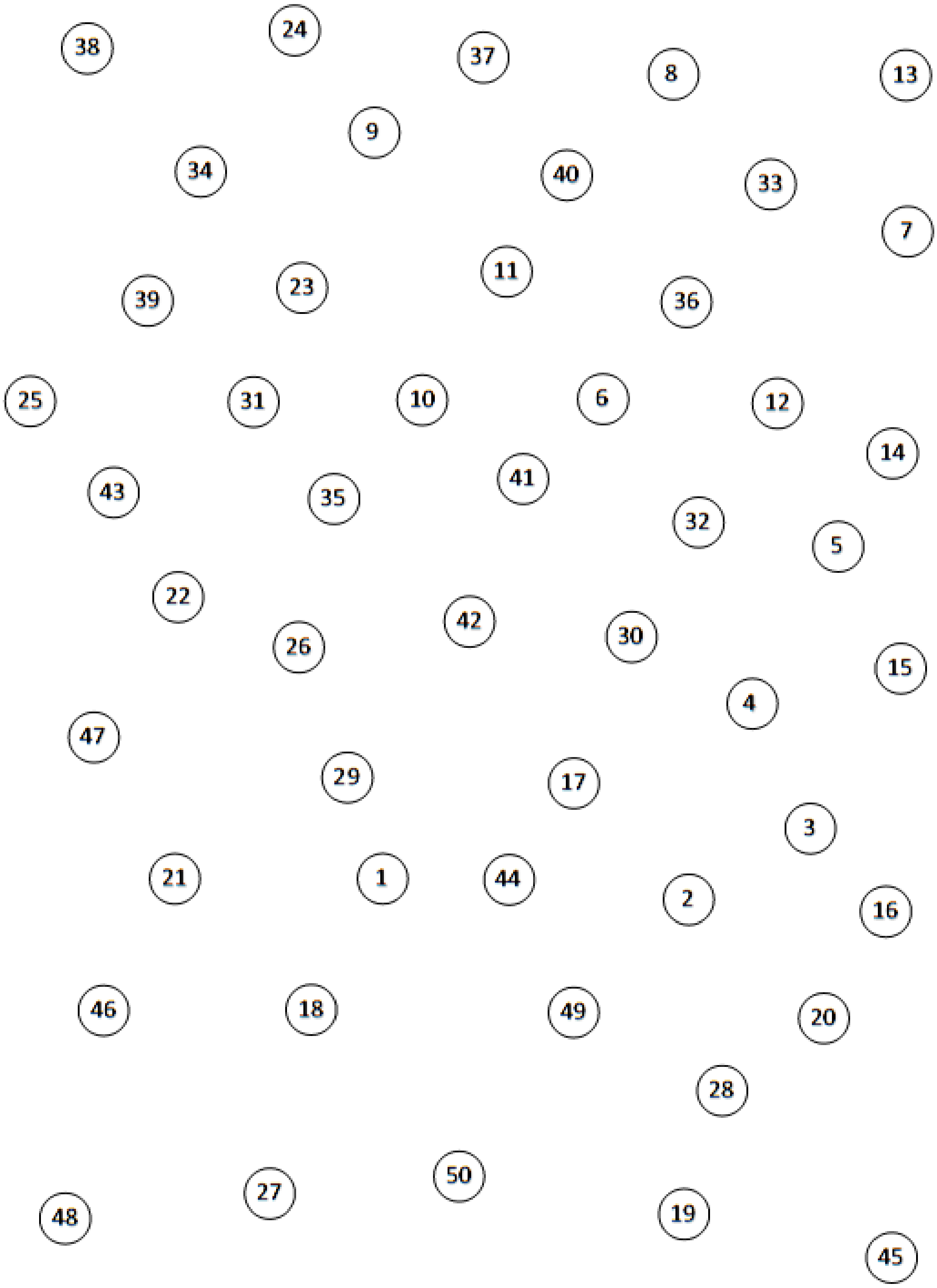

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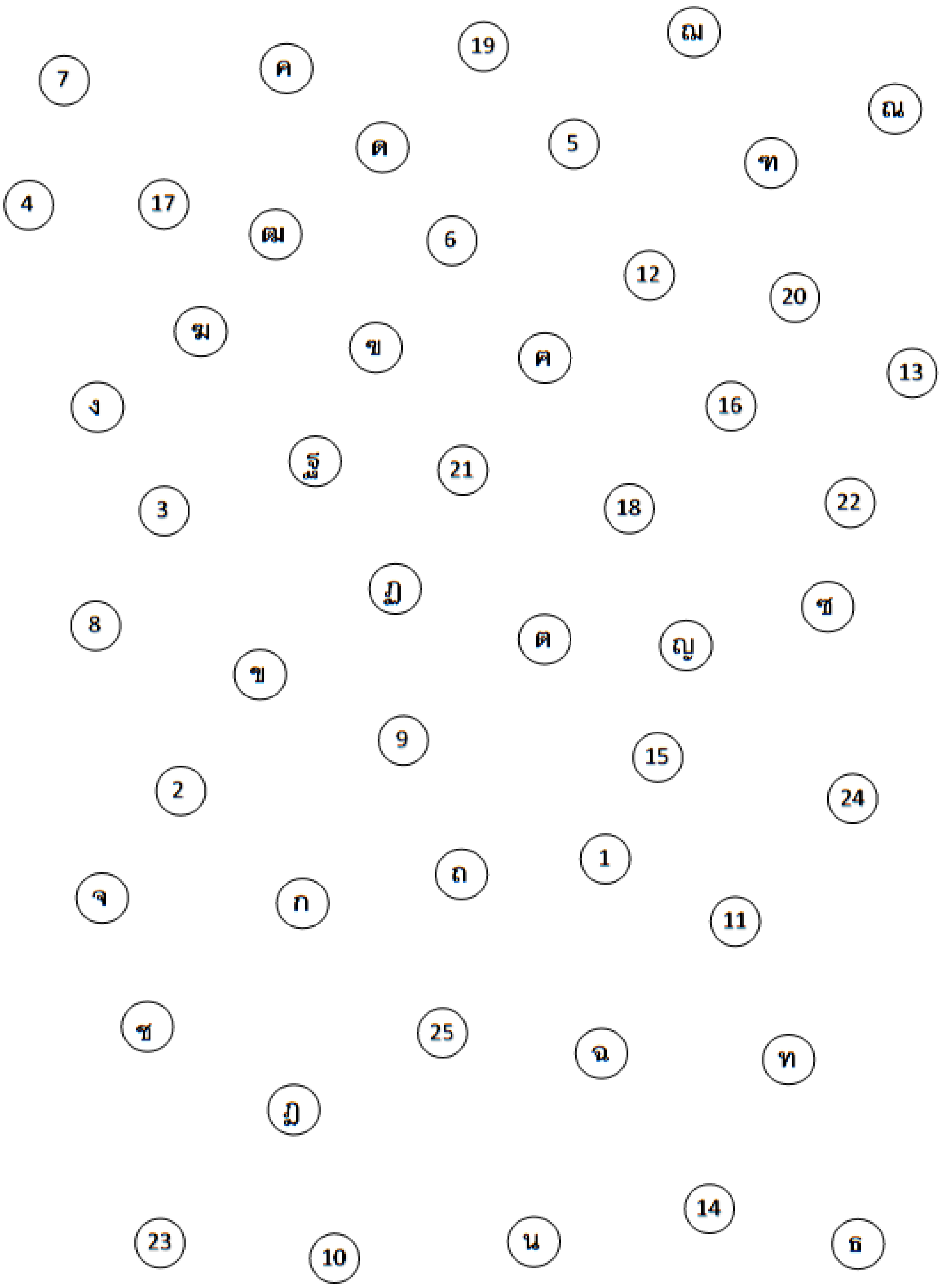

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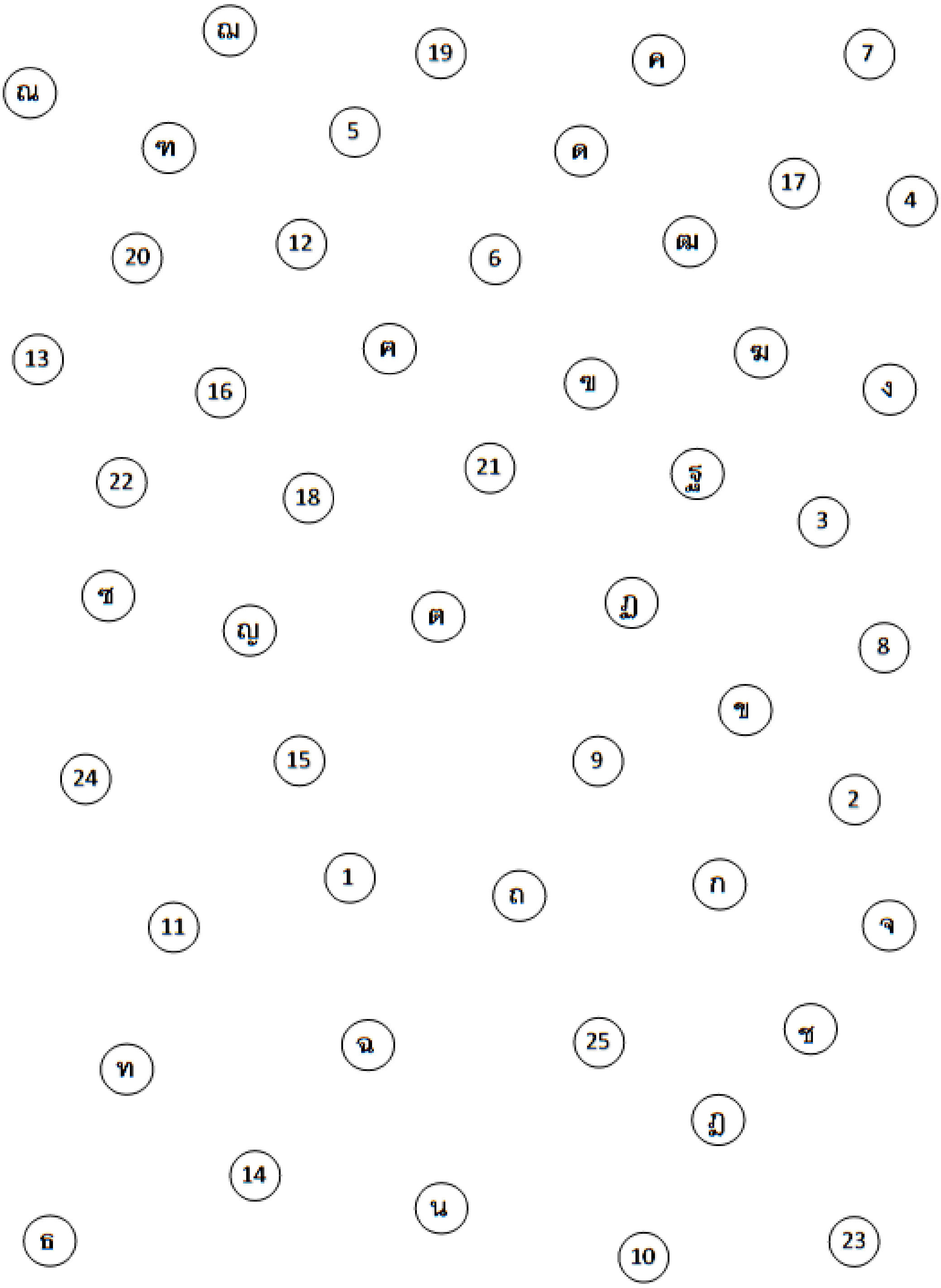
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23

Appendix G Stroop task

ห้อง..................เลขที่.......

Xs	เขียว	Xs	แดง	ชมพู	น้ำเงิน
เขียว	ม่วง	ม่วง	Xs	เหลือง	Xs
ม่วง	Xs	Xs	แดง	น้ำเงิน	เขียว
Xs	น้ำเงิน	ม่วง	เขียว	Xs	เขียว
ชมพู	Xs	น้ำเงิน	Xs	เหลือง	ม่วง
น้ำเงิน	ม่วง	Xs	แดง	ชมพู	Xs
Xs	Xs	ชมพู	ชมพู	แดง	น้ำเงิน
เขียว	ชมพู	แดง	Xs	แดง	Xs
Xs	น้ำเงิน	ม่วง	Xs	ม่วง	ชมพู
ม่วง	เหลือง	ชมพู	Xs	แดง	Xs
เขียว	น้ำเงิน	Xs	ชมพู	Xs	เหลือง
เขียว	Xs	เขียว	น้ำเงิน	ชมพู	Xs

ห้อง....................เลขที่.

น้ำเงิน	ม่วง	Xs	แดง	ชมพู	Xs
Xs	Xs	ชมพู	ชมพู	แดง	น้ำเงิน
เขียว	ชมพู	แดง	Xs	แดง	Xs
Xs	น้ำเงิน	ม่วง	Xs	ม่วง	ชมพู
ม่วง	เหลือง	ชมพู	Xs	แดง	Xs
Xs	เขียว	Xs	แดง	ชมพู	น้ำเงิน
เขียว	ม่วง	ม่วง	Xs	เหลือง	Xs
ม่วง	Xs	Xs	แดง	น้ำเงิน	เขียว
Xs	น้ำเงิน	ม่วง	เขียว	Xs	เขียว
ชมพู	Xs	น้ำเงิน	Xs	เหลือง	ม่วง
เขียว	น้ำเงิน	Xs	ชมพู	Xs	เหลือง
เขียว	Xs	เขียว	น้ำเงิน	ชมพู	Xs

Appendix H Verbal fluency task

ห้อง....................เลขที่...

คำที่ขึ้นต้นด้วย อักษร ง

คำที่ขึ้นต้นด้วย อักษร ม

คำที่ขึ้นต้นด้วย อักษร อ

ห้อง..................เลขที่....

คำที่ขึ้นต้นด้วย อักษร น

คำที่ขึ้นต้นด้วย อักษร ป

คำที่ขึ้นต้นด้วย อักษร ส

Appendix I DSST

DIGIT SYMBOL 1

No:________

1	2	3	4	5	6	7	8	9
—	⊥	⊐	L	⊔	○	∧	×	=

SCORE

SAMPLES

2	1	3	7	2	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3

6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7

9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

2	7	3	6	5	1	9	8	4	5	7	3	1	4	8	7	9	1	4	5	7	1	8	2	9

DIGIT SYMBOL 2

No: ____________

1	2	3	4	5	6	7	8	9
Λ	∟	○	⊐	—	=	×	⊥	⊔

SCORE

SAMPLES

2	1	3	7	2	4	8	2	1	3	2	1	4	2	3	5	2	3	1	4	5	6	3	1	4

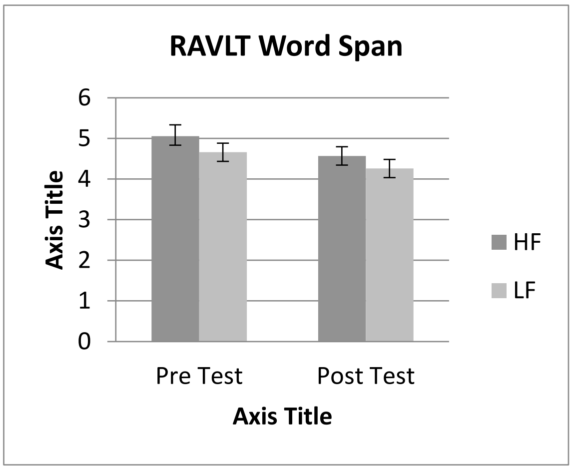
1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3

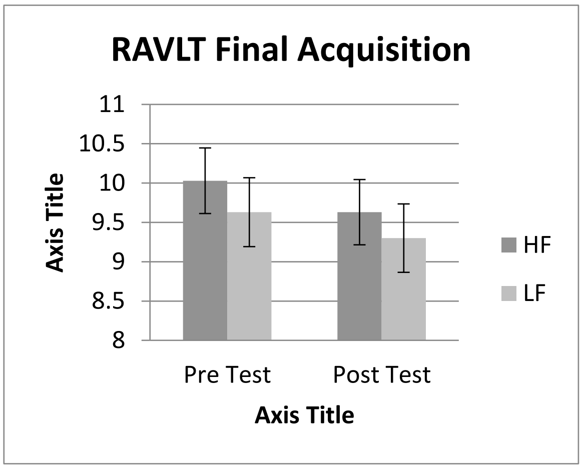
6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7

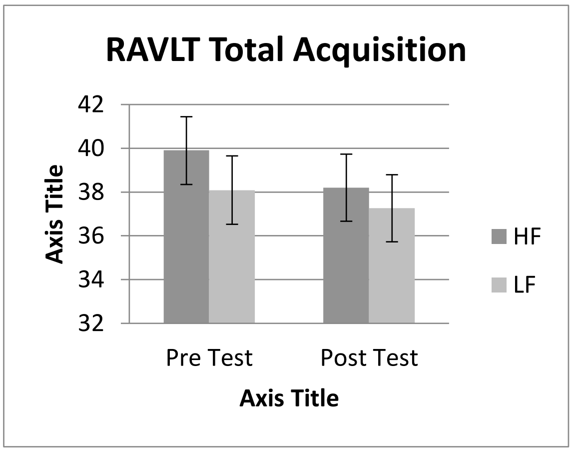
9	2	8	1	7	9	4	6	8	5	9	7	1	8	5	2	9	4	8	6	3	7	9	8	6

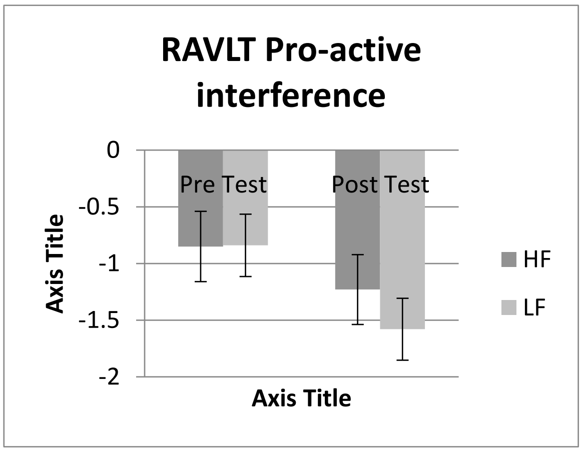
2	7	3	6	5	1	9	8	4	5	7	3	1	4	8	7	9	1	4	5	7	1	8	2	9

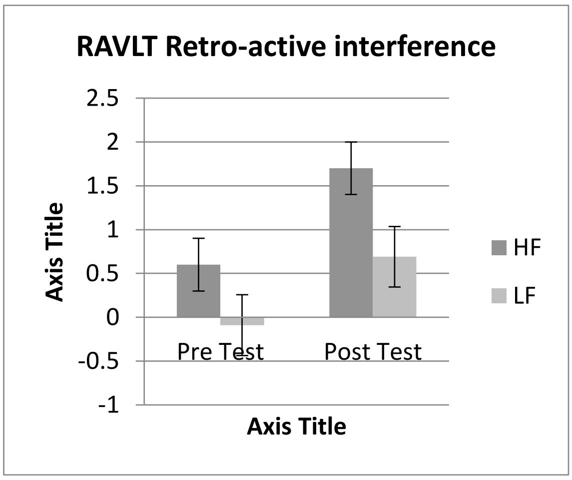
Appendix J Table of RAVLT variables

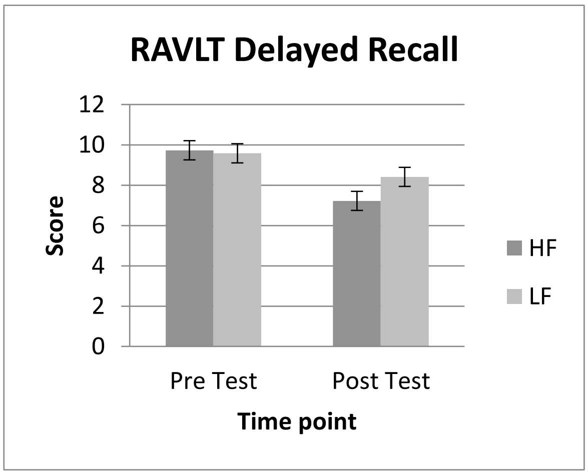


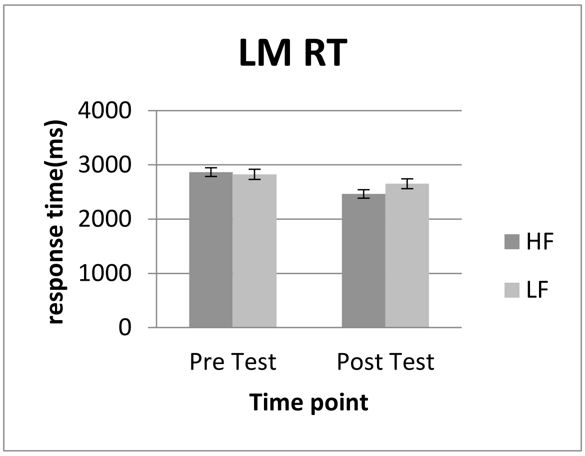


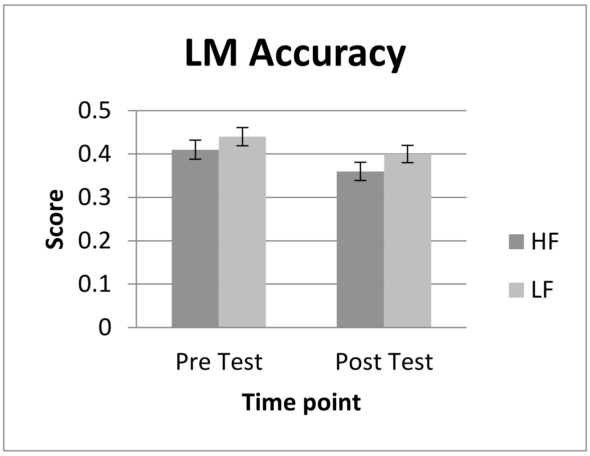


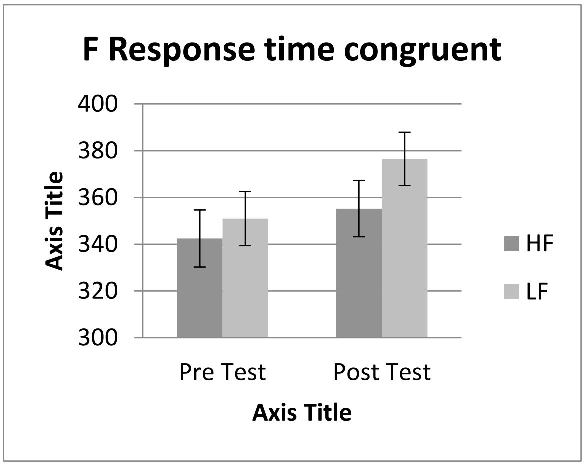


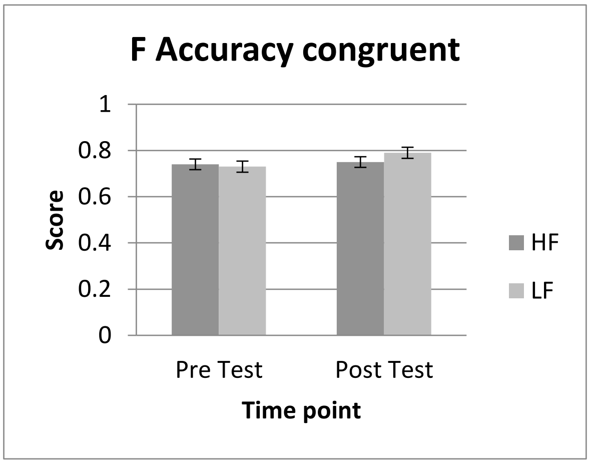


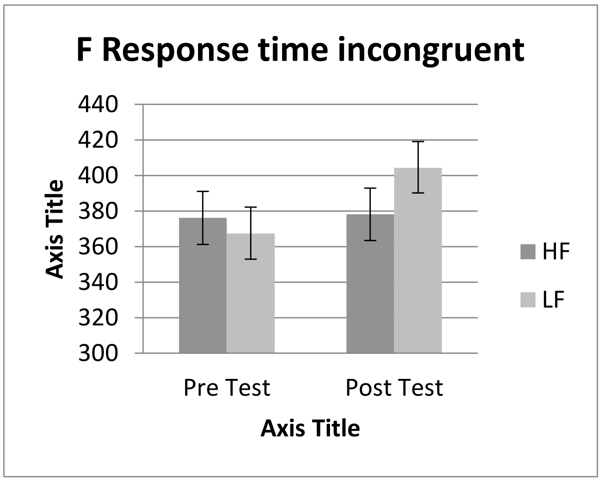


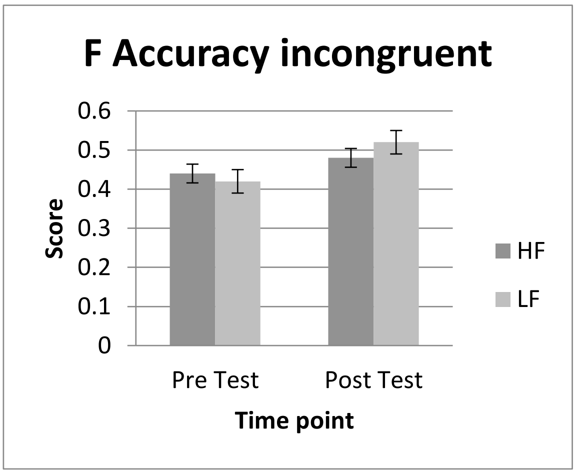

LM RT
response time(ms)
4000
3000
2000
1000
0
Pre Test
Post Test
Time point
HF
LF


LM Accuracy
Score
0.5
0.4
0.3
0.2
0.1
0
Pre Test
Post Test
Time point
HF
LF


F Response time congruent
Axis Title
400
380
360
340
320
300
Pre Test
Post Test
Axis Title
HF
LF


F Accuracy congruent
Score
1
0.8
0.6
0.4
0.2
0
Pre Test
Post Test
Time point
HF
LF


F Response time incongruent
Axis Title
440
420
400
380
360
340
320
300
Pre Test
Post Test
Axis Title
HF
LF


F Accuracy incongruent
Score
0.6
0.5
0.4
0.3
0.2
0.1
0
Pre Test
Post Test
Time point
HF
LF