The effects of omega-3 polyunsaturated fatty acids on the cognitive neuroscience of ADHD

Laura Alice Sellick

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Swinburne University

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Abstract

This thesis explored the effects of dietary supplementation with essential fatty acids on cognitive performance, cardiovascular function and brain activation in young adults, both with and without attention deficit hyperactivity disorder (ADHD). Essential fatty acids (EFA) play an important role in the body, from a broad dietary perspective to a cellular perspective, and have been shown to influence higher order functioning such as cognition, cardiovascular performance, and brain activation. While there is considerable behavioural research on the effects of certain EFAs on humans, there is comparatively little research that has investigated the effects of EFAs on cognitive function and brain function in young healthy adults. The majority of research focuses on ageing or clinical populations. Similarly, the literature on ADHD is largely confined to child groups and suggests the disorder is related to low EFA levels, lower cognitive performance and poorer cardiovascular health outcomes.

This thesis utilised a randomized, placebo-controlled, double-blind clinical trial to examine the effects of EFA supplementation on cognitive performance, cardiovascular performance and brain activation on young adults with and without ADHD (n=98). Participants were randomly allocated to one of three treatment groups (placebo, EPA-rich or DHA- rich) over a 12-week supplementation period. Such a process required the analysis of essential fatty acid levels in the two participant groups as well as establishing whether there were differences at baseline in terms of cognitive performance for tasks involving perception, attention and memory, and in brain responses, as measured by fMRI, to such tasks.

As expected, there were differences in EFA blood level changes between the supplementation groups, providing evidence of compliance with the supplementation regimens. Similarly, there were significant differences found in ADHD symptoms between the control and ADHD groups, but not between the supplementation groups. There were no differences between ADHD and Control participants in EFA blood level changes observed between time points.

Overall, while there were no cognitive differences between groups after supplementation, there were some differences observed in the pattern of differences between the two time-points of observation. Some differences were detected between the time points, with EPA-rich supplementation leading to more improvements than DHA-rich supplement. This thesis found few cardiovascular differences between the ADHD and Control participants or the supplementation groups either at baseline or after supplementation. This may be explained by the relatively young and healthy population who may have not yet begun to suffer age-related cardiovascular issues.

The fMRI results of this study suggest that patterns of neural activity while completing the Stroop task differed between ADHD participants and control participants at baseline. Control participants displayed less activation in the ACC (anterior cingulate cortex) and IFJ (inferior frontal junction) than ADHD participants, with no significant behavioural differences detected. This was explained using the theoretical framework of "neural efficiency" as a means of relating brain activity and behavioural performance. Initial analyses did not indicate an effect of EFA between Control and ADHD participants, but a post-hoc correlational analysis of behavioural reaction time data and BOLD activation suggests there are between group differences in this relationship. Further analyses on supplementation groups showed between group differences in the relationships between BOLD response and behavioural measures. It should be noted that these analyses were exploratory in nature. Limitations in this theory are discussed, and ways to extend the theory are explored within this thesis.

In conclusion, this thesis provides evidence that omega-3 supplementation may alter neurocognitive performance in young adults. In terms of brain activation, this thesis provides further evidence for the theory of neural efficiency demonstrated through the differences detected between control and ADHD participants

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Statement of Authorship

This is to declare that this thesis contains no material which has been accepted for the award to the candidate of any other degree or diploma, except where due reference is made in the text of the examinable outcome.

To the best of my knowledge this thesis contains no material previously published or written by another person except where due reference is made in the text of the examinable outcome; and where the work is based on joint research or publications, discloses the relative contributions of the respective workers or authors.

Laura Sellick

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Chapter 1 Introduction

1.1 General Introduction

A balanced diet is a vital aspect of health and well-being and an important part of a balanced diet is the inclusion of many sources of essential fatty acids (EFAs), a specific type of acid that must be ingested though dietary sources. Over time the staple diet of humans has changed, with rapid changes in the last 150 or so years as a result of the industrial revolution (Cordain et al., 2005). The lack of dietary sources of essential fatty acids has led to an increase in the incidence of physical and psychiatric disorders and illnesses such as obesity (Simopoulos, 2016), cancer and general health issues. One disorder that has been linked with essential fatty acid deficiency is attention deficit hyperactivity disorder (ADHD). Multiple research studies have investigated the fatty acid profiles of ADHD sufferers (Richardson & Puri, 2000; Stevens et al., 1995). While the evidence is not yet conclusive, it suggests that people with ADHD have poorer fatty acid levels than their control counterparts (Stevens, Zentall, & Burgess, 1996; Stevens et al., 1995). This thesis will investigate the relationship between EFAs and ADHD using a randomised, double-blinded clinical trial.

Nutritional intake plays an important role in the health and well-being of humans. Dietary intake has an impact on many areas of health including, but not limited to, cognitive performance, cardiovascular function and conditions such as diabetes and obesity (Simopoulos, 2016). Dietary intake has also been linked to other measures of health, such as mood and well-being (J. Lee et al., 2015; McMillan, Owen, Kras, & Scholey, 2011). As dietary intake is an important aspect of human health, it is imperative that research is conducted to know more about these nutrients, and how they interact with bodily function and performance.

Consumption of fresh foods such as fruits and vegetables is on the decline, being replaced by foods of convenience, lowering the typical levels of EFAs. This pattern of low essential fatty acids has been shown in a variety of different samples and cultural groups. While much data derives from the U.S.A, similar trends have also been reported in samples from Belgium (Staessen, De Bacquer, De Henauw, De Backer, & Van Peteghem, 1998) and Denmark (Bang, Dyerberg, & Sinclair, 1980). In the past, during the Palaeolithic period, diets were composed of a variety of unprocessed, natural foods, such as green leafy vegetables, fresh fish and a variety of nuts, grains and seeds. These foods that formed part of the staple diet had high levels of nutrients, and were conducive to good health in times of plenty. Though some communities may have had limited access to fish due to living in

locations that were geographically far from the coast, such communities were eating other types of lean meat and vegetables.

If society were to consume an optimal level of fish in their diet, then supplementation would not be necessary, but research shows that a typical modern Western diet does not contain enough fish to fulfil dietary recommendations of essential fatty acids (Simopoulos, 2008). These deficits may be caused through low levels of consumption of the nutrient or through total omission of the nutrients from dietary intake. Fish oil can be obtained in a variety of different ways, with common sources including salmon, sardines, mackerel and other types of fatty fish. Commercial fish oil companies make many claims on their product labels, including healthy joints, cardiovascular health, brain function and memory improvement, alongside many others. However, very little of the human clinical research has been conducted on a young healthy adult population. This thesis aims to address this issue.

Research has suggested a high percentage of people (68%) use some source of dietary supplementation in the Western population (Council for Responsible Nutrition, 2012). There are many reasons why people use supplements, including poor typical nutritional intake and convenience. Certain supplements are designed to improve specific aspects of health such as the cardiovascular system, healthy joints and brain function and development. The marketing of these products claims they have beneficial effects on many aspects of human health. These supplements are designed to restore the body to function to its full potential, and include examples such as multivitamins and fish oil. Regarding fish oil, the supplementation of omega-3 polyunsaturated fatty acids (n-3 PUFAs) is particularly popular, and there is a large body of literature investigating the effects of omega-3 supplementation on a wide variety of areas, including cognition, brain function and cardiovascular performance. Despite the high usage of these supplements and the claims of their manufacturers, more research is needed to investigate their effects on the systems they claim to help.

Although there is already quite a substantial amount of research conducted on the effect of omega-3 levels on brain performance and cardiovascular performance, the majority is focussed on a relatively selective demographic. Most research studies have focused on either children, whose brains are still in rapid development (Richardson & Montgomery, 2005), or on older participants in relation to age-related cognitive decline (Dangour et al., 2010). Of the little research that is done in the ages in between, most has been conducted on a clinical sample. This indicates there is an area of research worth addressing, as there are many opportunities to be gained by investigating a healthy young adult population as

a sample for the study. This thesis will explore whether results in a young adult sample with and without ADHD portrays the aforementioned trends, and investigate possible reasons for this. Such comparisons will be conducted on those 18 through to 40 years of age, to help establish a greater amount of literature on those that are not affected by age-related cognitive maturation or decline. If an improvement in these variables can be associated with better essential fatty acid status in a young healthy population, it is more likely to be due to a direct effect than if it was observed in an ageing population.

By using a sample of young adults, it can be expected that this subset of the population is at their cognitive peak. While crystallised intelligence stays reasonably stable throughout the ageing process, fluid intelligence has been found to decline over the lifespan (Bugg, Zook, DeLosh, Davalos, & Davis, 2006), with this decline beginning quite early while people are in their early to mid-20's. Past their maturational development, but optimally distant from age-related cognitive decline, this sample provides an interesting insight into the role of diet and supplements on the human condition. Additionally, as they are less likely to suffer from possible confounding variables such as heart disease or chronic illnesses, any changes in performance are more likely to be able to be attributed to diet and/or supplementation than their elderly or middle-aged counterparts.

While essential fatty acid status influences numerous bodily systems, this thesis will focus on its effects on cognitive performance, cardiovascular health and brain function and will investigate the effects of EFAs on these systems and the implications of these findings. EFAs have been found to have an effect on cognition, with essential fatty acid levels having been linked to the prevention of cognitive decline (Assisi et al., 2006). Brain cells are especially rich in long chain polyunsaturated fatty acids PUFAs of the n-3 and the n-6 type (Lunn & Theobald, 2006) which are necessary for the normal growth and development of the brain (Gomez-Pinilla, 2008). As the brain cells are rich in these acids, it has been suggested that PUFAs may play a fundamental role in cognitive function (Lunn & Theobald, 2006), and therefore a lack of these nutrients in staple dietary intake may lead to an impairment in cognitive performance. In addition to cognitive performance, EFA status has also been linked with cardiovascular performance, with higher EFA levels being associated with better cardiovascular performance both epidemiologically (Bang et al., 1980) and clinically (Bucher, Hengstler, Schindler, & Meier, 2002; Marchioli, 1999). As cardiovascular disease has such an impact on society, it is important that research is conducted in this area.

This thesis aims to examine the effect of dietary intake of essential fatty acids on cognitive, cardiovascular and neural responses in a sample of young adults, both with a control

sample and a sample of those suffering from ADHD. A double-blinded, placebo controlled study design was designed to investigate the effect of EFA levels on cognitive, cardiovascular and neural parameters at baseline and also after 12 weeks of fish oil supplementation. It investigated which essential fatty acid is the best to use in supplementation. The majority of fish oil supplementations contain both EPA and DHA omega-3 PUFAs; however, it is not understood whether one is more advantageous than the other, or a combination of both is ideal. Many studies have looked at EPA and DHA in isolation, but only a small number have directly compared the two, a feature which will be further discussed in later chapters.

1.2 Thesis Structure

This thesis consists of 11 chapters, as detailed below.

Chapter 2 - Essential Fatty Acids

Chapter 2 explains common sources of fat in the diet, and the function of fats in the human body. It begins by addressing the importance of diet on general health, and changes in the staple diet of humans over time. It addresses the issue of current dietary intakes, the changes that have occurred, as well as many of the factors which have led to the need for supplementation in order to have an optimal intake of EFAs. It also discusses the health effects that supplementation and the subsequent optimal levels provide, as well as looking at the sources of these nutrients, and in which ways these specific food types are present in the human diet. It then specifically focusses on essential fatty acids that form the focus of this thesis. By defining EFAs and outlining the different types of these, this chapter explores these acids, their chemical properties, synthesis, metabolism and function. By showing the importance of their presence on the health and well-being of humans, this chapter highlights the necessity of research in this area.

Chapter 3 - Effects of EFAs

Chapter 3 discusses the effect that changes to EFA levels may enable, both at an epidemiological and clinical level. It is important to look at essential fatty acid status without supplementation at an epidemiological level to get an indication of baseline nutritional levels using a sample of the population. As it is not practical or possible to supplement the entire population, it is interesting to see how dietary intake at a population level affects health. However, as outlined earlier, dietary intake of these acids is not adequate in the majority of the modern Western population. Results from clinical trials help to show the effects of acids in a controlled environment. Chapter 3 explores the literature pertaining to the effect that EFAs can have on different parameters including

cardiovascular health, cognitive performance and neural efficiency from both an epidemiological and clinical perspective. By reviewing the literature on randomised control and clinical trials, it investigates whether EFAs may improve the health of the population.

Chapter 4 - ADHD

Chapter 4 explores ADHD as a disorder, with a particular focus on the manifestation of the disorder in adults. By doing this, it builds the readers understanding of the disorder and explains how this research fits into the bigger picture. It will look at the history of the disorder, how it has changed over time, and the impact of these changes. By addressing the controversies associated with adult ADHD, it highlights the need for more research conducted on young adults. With this in mind, there is a vast amount of literature on EFA supplementation pertaining to children with ADHD, and comparatively little on adults. Nevertheless, because evidence suggests that the symptoms of ADHD continue from childhood, through adolescence and into adulthood, the larger literature on EFA supplementation can help guide results for adults. Therefore, this thesis also aims to expand on the small amount of literature on ADHD in adults.

As outlined earlier in the thesis, it has been hypothesised that essential fatty acid status is related to the prevalence and symptomology of ADHD. This thesis will investigate the connection between essential fatty acid status and ADHD. This literature review will aim to provide the reader with a well-rounded view on the relationship between EFA status and ADHD. In doing so, the chapter will outline why EFA supplementation may be a viable treatment option for ADHD, along with the traditional pharmacological and psychological treatment methods.

Chapter 5 - Methodology

Chapter 5 consists of a detailed description of the methodology used in the studies that make up this thesis, and will provide a lead in to the following experimental chapters. The experimental chapters contained in this thesis adopt a clinical trial methodology, and chapter 5 outlines what this methodology consists of, the advantages and limitations of the methodology and why it was chosen for this thesis. It will provide an explanation for each task utilised and justify their use in the project. By providing an understanding of the methodology used, it will make it easier for the reader to understand future chapters and focus on the experimental content.

Chapter 6 - Baseline results

Chapter 6 is the first experimental chapter in this thesis. It will present the findings of a baseline analysis of differences in essential fatty acid status. There are many aspects investigated in this chapter, including the general effect of EFA status on cognitive performance and cardiovascular functioning in young adults. The first part of this chapter focusses on the young adults as an entire group, not distinguishing between the control participants and participants with ADHD. It investigates the EFA blood levels of these participants and if they replicate previous findings in the literature with regard to baseline EFA levels. It examines whether there are any general baseline effects of essential fatty acid status, cognitive performance and cardiovascular function. It then looks if there are any differences between the healthy controls and the participants with ADHD. Literature has suggested that sufferers of ADHD have different EFA levels (Antalis et al., 2006). By looking at whether differences occur, the thesis investigates whether the data complements the literature or not.

Chapter 7 - Bloods

This chapter will provide information about the uptake of the EFAs between the groups, and has many aspects to investigate. By comparing the difference in EFA levels of participants with ADHD with that of matched controls, this research will investigate whether there is a difference in uptake between the two groups. As both groups are on the same supplementation, any difference in changes of levels may be attributable to the disorder. Many other factors such as compliance, dietary habits and others are also involved, but the use of a matched population will minimise the effect of extraneous variables. Chapter 7 will also investigate EFA levels between supplementation groups to determine the uptake of different EFA formulations, also providing a compliance check to ensure the supplementation groups levels of EFAs change and the placebo groups levels do not change.

Chapter 8 - Psychophysics/Cognition

Chapter 8 will investigate the differences in cognitive performance after the three month supplementation period. A body of research suggests that blood levels of essential fatty acids may affect cognitive performance (Fontani et al., 2005; Lunn & Theobald, 2006). This chapter will determine whether these findings are replicated in this sample group, and if so, whether there is a difference in performance between participants with and without ADHD.

Chapter 9 - Cardiovascular function

Chapter 9 investigates the effect of essential fatty acid supplementation on cardiovascular function. A body of research suggests that blood levels of essential fatty acids may affect cardiovascular function (Bang et al., 1980; Bucher et al., 2002). This chapter will determine whether these findings are replicated in this sample group over a 12 week supplementation period, and if so, whether there is a difference in performance between participants with and without ADHD.

Chapter 10 - fMRI

Chapter 10 uses functional magnetic resonance imaging to investigate brain function in young adults with and without ADHD. It examines whether there are any initial differences between the brains of ADHD and controls in terms of performance on a Stroop task. It then examines the effect of essential fatty acid supplementation on brain function, both between control and ADHD participants and between the three supplementation groups. The major focus of this chapter is to investigate whether the neural efficiency theory (Haier et al., 1988), which asserts that higher cognitive performance is associated with lower levels of brain activation, can explain the findings of the study.

Chapter 11 - Discussion and conclusion

Chapter 11 outlines and discusses the major findings of the thesis in light of the current literature. It provides a link between the results of each study and how this fits in to the research as a whole. Comparisons between pre and post treatment will attempt to investigate generic changes associated with n-3 absorption, cognitive and cardiovascular performance, as well as between those with and without ADHD. This chapter addresses each of the hypotheses presented earlier in the thesis and establishes whether they were supported or not. Through exploring the outcome of these hypotheses, it will provide a clearer picture of the all the results and what the findings mean in terms of the current literature published. It will also conclude the thesis by providing a summary of the overall findings and their relevance to the research community, by relating the findings of this study to past and present literature. It will also identify the limitations of the research and suggest ways to address these. It will look the real world implications of this research. This will be looked at both in terms of dietary and lifestyle recommendations and other findings that were made. Additionally, it will identify future directions in the field and suggest ideas for further research. By using the results found in this thesis, future research paths can be suggested which would give a further understanding of the effect of EFAs on cognition and cardiovascular function, both in a young adult ADHD population and an age and gender matched control sample.

Chapter 2 Essential fatty acids

2.1 Chapter Overview

Essential fatty acids play an important role in the body, from a broad dietary perspective to a cellular perspective and have been shown to influence higher order functioning such as cognition as well as cardiovascular performance (J. Cohen, Bellinger, Connor, & Shaywitz, 2005; Fontani et al., 2005; Wijendran, 2004). The purpose of this chapter is to provide an overview of omega-3 fatty acids by explaining what they are, their purposes in the human body, and the biological mechanisms in which they play a part. By providing the reader with an understanding of the concept of fats, and more specifically essential fatty acids, this chapter will outline their functions in the body and why they are important in a healthy lifestyle.

2.2 The Importance of Diet on Overall Health and Wellbeing

This chapter will also discuss the many dietary changes over history and use epidemiological evidence to support the importance of diet. As discussed in the preceding chapter, human health is influenced by many factors, some determined by genetics and others determined by the environment. The exact interaction of the environment and genetics is not yet fully understood; however it is well accepted that both of these factors have an influence on human health. There are many types of environmental influence, but diet is a major contributing factor. More importantly for our wellbeing potential, while humans have little control over their genes, dietary intake is within our control. A balanced diet is a vital aspect of health and well-being, and contributes to this as a whole. One important factor in a balanced diet is the inclusion of an adequate supply of essential fatty acids (Cordain et al., 2005), and this thesis aims to investigate the effect of these on cognitive performance, cardiovascular health and brain function.

If we compare the diet of the Palaeolithic human to one of the modern human, it can be seen that there are many differences, including in the levels of EFAs (Simopoulos, 2006). These dietary modifications have not only resulted in changes in the absolute values of these acids, but also their ratios. Given essential fatty acids are acknowledged as an important part of a balanced diet (Cordain et al., 2005), an imbalance is likely to have consequences on overall health. Through investigating the relationship between lower levels of EFAs in the diet and the increased incidence of chronic diseases in the same timeframe (Simopoulos, 2004), this chapter will review the importance of essential fatty acids in the diet, and show how deficiencies in them can be linked, in certain cases, to chronic disorders.

A balanced diet has an important influence on the health and well-being of humans. By consuming adequate amounts of the required nutrients, humans can maintain good health and prevent health issues such as cardiovascular disease and obesity from developing, lessening the burden on health care systems (Riediger, Othman, Suh, & Moghadasian, 2009). As there is no single food group that contains all of the required nutrients, it is important that humans consume food from all of the different food groups in order to maintain a balanced diet. It must be noted that staple diets vary across the world, and this may create differences in nutrients consumed. However, there are basic guidelines that are universal across the globe. Thus, the World Health Organisation (WHO) suggests that humans limit energy intake from total fats, consume more unsaturated fats and less saturated fats and try to completely eliminate trans-fatty acids from the diet (WHO, 2013). These guidelines, when combined with regular physical exercise, are also very similar to the dietary recommendations of the American Heart Association (Lichtenstein et al., 2006) . If these recommendations are followed, evidence suggests that they have been found to lower the risk of heart disease and stroke and help people reach and maintain a healthy body weight, all of which can be seen to benefit human health (Krauss et al., 2000).

2.3 Fats

Functions of fats

Lipids serve a number of functions in the human body. They form a vital part of cell structure, and serve an important role as energy reserves by providing a high amount of energy to the body, roughly twice as much as carbohydrates gram for gram (Martini, Nath, & Bartholomew, 2008). They also play an important structural role by forming the main part of cell membranes (Martini et al., 2008). They help to maintain an appropriate body temperature and provide a protective barrier against bodily trauma (Martini et al., 2008).

Categorisation of Fats

Fatty acids (FAs) are carboxylic chains that end in a methyl aliphatic tail. There are several different categories of fatty acids, with approximately 20 different types consumed within the typical human diet. In the human diet, fatty acids are either in triglyceride form or free fatty acid form. Triglyceride fatty acids are an ester derived from glycerol and three fatty acids. Free fatty acids occur as a result of the process of the breakdown of the triglycerides.

Fatty acids are composed of hydrocarbon chains contained within the body, made up of a carboxyl group (COOH) at one end, and a methyl group (CH3) at the other end known

as the omega end. Fatty acids are composed of an even number of carbon atoms, ranging from two to thirty. The distinction between the types of essential fatty acids lies in the placement of the carbon to carbon double bond relative to the placement of the omega molecule (Lunn & Theobald, 2006). Hence, if the first double bond is located six positions from the methyl end, it is known as an omega-6, and if it is three positions from the methyl end, it is known as an omega-3.

The chemical structure of fatty acids can be recognised by three numbers used to represent its chemical composition, for example EPA is often represented as 20:5 n-3. The first number of this number refers to the length of the carbon chain and the second number refers to the number of double bonds present in the molecule. The final number is indicative of the number of carbons to the first double bond counting from the omega end. Hence, EPA contains 20 carbon atoms, has 5 double bonds and is an omega-3 fatty acid.

Fatty acids are broken down into two main categories, saturated and unsaturated fatty acids, with the presence of carbon to carbon double bonds being the distinguishing feature between the two categories. Saturated fats do not contain any carbon to carbon double bonds, and are quite rigid in structure. Unsaturated fats are characterised by the presence of at least one carbon to carbon double bond. If an unsaturated fat only contains one carbon-carbon double bond, it is termed mono-unsaturated. If an unsaturated fat contains more than one carbon-carbon double bond, it is termed a polyunsaturated fatty acid (PUFA). Therefore, a PUFA is defined as a fat that contains more than one carbon-carbon double bond.

While a variety of fats is necessary for a balanced diet, an important part of dietary recommendations is the inclusion of essential fatty acids. Most fatty acids can be created within the human body through the use of enzymes which are naturally sourced from the human body. There are two types of PUFAs that cannot be naturally synthesised (de novo) by the human body. These PUFAs are hence termed “essential fatty acids” and are differentiated from other families of PUFAs as they must be obtained through dietary sources or nutritional supplements. These are omega-3 linolenic (n-3) acids and omega-6 linoleic (n-6) acids. Essential fatty acids are abundant in fresh oily fish, and are also found in other sources such as green leafy vegetables, tofu and certain nuts. As these foods only form a small proportion of the typical modern Western diet, the risk for deficiencies in these nutrients is high.

Two important essential fatty acids are alpha-linolenic n-3 fatty acid (ALA) and linoleic n-6 fatty acid (LA), as these are the precursors for the n-3 and n-6 PUFA acids. These are

known as parent fatty acids, as all biologically active long chain n-3 and n-6 acids are derived from these two fatty acids.

ALA is the precursor for omega-3 PUFAS, and the main metabolites created from these acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as docosapentaenoic n-3 acid (DPA n-3). This synthesis occurs though the precursor fatty acids undergoing a series of elongation and desaturation processes (Brenna, Salem Jr, Sinclair, & Cunnane, 2009). LA is the precursor for arachidonic acid (AA) and docosapentaenoic n-6 acid (DPA n-6). This synthesis also occurs via a series of elongation and desaturation processes (Brenna et al., 2009).

Despite the conversion pathways for n-3 and n-6 fatty acids being entirely independent from each other, they follow the same pathway and require the same enzymes at each step. Additionally, both omega-3 and omega-6 fatty acids compete for the same position on the phospholipid membrane (Burdge & Calder, 2005). This puts them in opposition with each other to convert, and hence they have a competitive relationship (Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001). Past research has found that the enzymes required to desaturase show a preference for the ALA to DHA, giving the n-3 a preferential likelihood over the n-6 fatty acids (Brenna et al., 2009). As dietary intake is the only source of PUFAs, this suggests that dietary intake determines which of the fatty acids is able to utilise the required enzymes and therefore convert into its longer chain constituents. Due to the preferential treatment of omega-3 elongation process (Brenna et al., 2009), the greater the omega-3 dietary intake is, the lower the amount of omega-6 fatty acid chains present.

Sources of PUFAs

Omega-3

In order to adequately function, the body needs a balance of n-3 and n-6 fatty acids (Lunn & Theobald, 2006). However, research has shown that despite the need for a balance between these fatty acids in the diet, Western diets are typically low in omega 3 acids and overly high in omega 6 acids (Muhlhausler & Ailhaud, 2013). It has been suggested that current diets may not contain an adequate nutritional intake, particularly in the area of essential fatty acids (Lunn & Theobald, 2006).

In terms of dietary intake, omega-3 fatty acids can be found in a variety of sources. Short chain omega-3 fatty acids, such as ALA, are found in an abundance of food products, including canola oil, certain nuts, tofu and green leafy vegetables. Long chain omega-3 fatty acids, such as EPA and DHA, are harder to obtain than their short chain counter parts

through dietary sources, but are still readily available in a properly balanced diet. These fatty acids are produced by micro-organisms such as algae which are eaten by the fish and are then passed on through the food chain (Lunn & Theobald, 2006). The best sources of omega-3 fatty acids are in fresh oil-rich ocean fish, such as sardines, salmon and mackerel.

While the best source of these acids is through oily fish, they are also present in plant sources such as flaxseed and canola oil. These plant sources are rich in ALA, a metabolic precursor for EPA and DHA. After the food sources of these nutrients have been ingested, the body attempts to convert the ALA into its long chain constituents of EPA and DHA. This occurs through enzymatic processes that occur in the endoplasmic reticulum of liver cells (Scott & Bazan, 1989). While this conversion of ALA into EPA is technically possible, the process of conversion is very inefficient in humans (Burdge & Calder, 2005). Conversion of ALA to its relevant long chain form is estimated at less than 5-10% for EPA and 2-5% for DHA (Davis & Kris-Etherton, 2003).

One of the reasons for this low conversion rate is that not all of the ingested fatty acids undergo the processes required to form long chain PUFAs. ALA is a substrate for beta oxidation in humans (Burdge & Calder, 2005), and if ALA is used in beta oxidation it reduces the amount present for conversion. The proportion of ingested ALA have been estimated through the method of labelled CO_2 present in breath (Burdge & Calder, 2005). It has been estimated that the fractional beta oxidation of ALA in women was 22% and 33% in men (Burdge, Jones, & Wootton, 2002; Burdge & Wootton, 2002). These values are quite high, especially when considered that evidence suggests they may represent a 30% underestimate of the true values, due to some CO_2 being trapped in bicarbonate pools (Berger & Cassuto, 2014). As a result of this limited amount of ALA still being present for the conversion processes, the amount of EFAs yielded from these processes are even smaller than one might have imagined. As the conversion rate is so low, it can be seen that ALA does not provide adequate levels of long chain PUFAs in humans, and this highlights the importance of adequate dietary sources of long chain PUFAs, such as EPA and DHA, to keep the body at optimal function.

Why Do We Need to Supplement?

As dietary intake is the only source of these nutrients, it is important that humans consume a balanced diet in order to take advantage of the health benefits these nutrients provide. If all of the nutrients required by the human body are able to be obtained from dietary intake then one might ask why we need to supplement. Supplementation is necessary because the typical modern Western diet is typically lacking in foods rich in omega-3 fatty acids, making the rate of omega-3 deficiencies very high (Simopoulos,

1999). Research has indicated that 90% of an Australian adult sample are eating less than the recommended levels of omega-3 required to reduce the risk of chronic disease (Howe, Buckley, & Meyer, 2007). For this reason, people are turning to supplements to fulfil their nutritional requirements.

A dietary supplement is a preparation of concentrated nutrients, such as vitamins or minerals, which is intended to supply the body with these nutrients. Supplements are designed to be taken when the nutrient being supplemented is either completely absent from the diet, or if it is not being consumed at adequate levels. If a population is ingesting enough of these nutrients through dietary sources, then there would be no need for the population to be taking supplements.

Amongst the dietary supplements that have received increasing interest in recent years are the omega-3 polyunsaturated fatty acids. There are many different products that supplement omega-3 fatty acids, each containing different ingredients. These products claim to improve health in a variety of ways, with everything from joint health to cardiovascular health to brain development and brain function. However, there is some conflicting evidence on the effects of omega-3 fatty acid supplementation on brain function. This problem is further complicated by most research being conducted either on children, or on older populations with very little research being conducted on young, healthy control participants.

Changes Over Time

It can be seen from earlier in this chapter that dietary intake is the definitive factor in EFA levels. However, diets do not remain constant throughout history. Since the beginning of the agricultural revolution about 10,000 years ago, there have been significant changes in the staple diet of humans. Studies have suggested that the content of the human diet has changed over the last 150 years in particular, with major changes in the type and amount of fats consumed and vitamin intake (Simopoulos, 2002).

Before the agricultural revolution, people used to eat what was available to them, primarily unprocessed whole foods (Cordain et al., 2005). Staple foods included plant and animal products such as lean meat, fresh fish, leafy vegetables, fruits and nuts. Humans also ate a large variety of wild plants, dependent on the climate and location of where they lived. While some communities may have had limited access to fish, their diet consisted of what was available to them at the time in the area where they resided.

As time progressed, the domestication of plants and animals became widespread, causing the characteristics of these staple foods to change. This was slow and subtle at first, but

accelerated with the development of new technologies (Cordain et al., 2005). This changed dietary patterns in two main ways. Firstly, it reduced the amount of healthy foods which were consumed in the diet. Secondly, it also introduced foods that did not exist as part of the pre-agricultural revolution diet such as dairy products, cereal grains and refined sugars (Cordain et al., 2005; Cordain et al., 2000). These foods did not provide as much nutritional benefit as the natural and unprocessed food eaten by our ancestors but they began to form a staple part of the modern western diet. 72.1% of the total energy consumed by people in the U.S is obtained from foods that would not have contributed any energy in the typical pre-agricultural diet (Cordain et al., 2005). This rapid shift in dietary intake began to have an adverse effect on human health.

One of these major changes in dietary patterns in the past 10,000 years is the reduction in essential fatty acids from the diet. Cordain et al. (2005) suggested that the change in staple foods caused a major change in the characteristics of seven key nutrients of the diet, with one of these dietary indicators being identified as essential fatty acid composition. Fish and seafood consumption has steadily decreased since the agricultural revolution. Fatty fish are rich in polyunsaturated fatty acids. A chronic deficiency in these nutrients may not only lead to general health problems, but also may inhibit the full potential of the human brain and the cardiovascular system.

Additionally, the consumption of fruits and vegetables has also decreased over time. In the past humans ate a wide variety of plant based foods, whereas in today's modern diet 17% of plant species make up 90% of the world's food supply (Simopoulos, 2002). These dietary changes are due to many factors. In the past, people did not have a choice but to eat fresh fish and produce, due to there being no other viable options. Today, society has a large variety of food which a person can consume whilst avoiding fresh food. Healthy food often has a lengthy preparation time, and may be neglected in preference to easy to prepare convenience foods which often lack in nutrients. Another factor affecting this dietary change is the high monetary cost of fresh food compared to convenience foods. Whilst one may argue that this initial outlay may be offset by the prevention of future health problems, some people do not have the financial means to consistently purchase fresh food.

Lifestyle changes during this time have also had an effect on the typical diet of humans. The industrialisation of society and increased reliance on technology that has developed over the last century have led to profound lifestyle changes. People work longer hours than they used to, with 26.5% of men reported to be working 50 hours per week in 2000, up from 21% in 1970 (Jacobs & Gerson, 2004). These longer work hours have led to

people having less time to cook and prepare food, and there has been a turn to foods of convenience rather than nutritional needs. These foods of convenience may be takeaway food, typically high in saturated fats and salt, or food prepared by the consumer that may not contain adequate nutrients. The longer work hours have also lessened the amount of time people have to exercise, especially during the winter months when daylight hours are limited.

Research has shown that an inverse relationship exists between dietary fat intake and physical activity (Pate, Heath, Dowda, & Trost, 1996; Simoes et al., 1995). Consequently, it is important that other factors as well as diet are considered when looking at the health of a person. Another factor that may affect human health is sedentary lifestyle behaviours which have been found to be detrimental to human health (Hamilton, Hamilton, & Zderic, 2004; Tremblay, Colley, Saunders, Healy, & Owen, 2010). This has been evidenced by the observation of quantitative differences in biological processes dependent on whether physical activity was imposed (Hamilton et al., 2004).

The development of technology over the last century has led to changes in the workforce, with occupation related physical activity decreasing over time (Knuth & Hallal, 2009). For example, many jobs that used to be performed manually are now conducted by machines, and many people spend their work day in front of a computer. Additionally, the introduction of public transport options such as trains and buses, and private transport such as cars, have reduced the amount of physical exercise required in everyday life.

Stress levels are also high, possibly leading to an increase in smoking and alcohol consumption, both of which have been linked with the absorption and metabolism of serum fatty acids(J. A. Simon, Fong, Bernert Jr, & Browner, 1996). Research has found that after controlling for cholesterol levels, energy intake and dietary fat levels, smoking levels were associated with a lower ratio of polyunsaturated to saturated fatty acid when analysed using univariate analyses (J. A. Simon et al., 1996). It also revealed that smoking was independently associated with lower levels of DHA and AA (J. A. Simon et al., 1996). While these results do suggest an association, care must be taken when interpreting the results, as it is possible that the results are due to extraneous variables. As differences in dietary patterns have been detected between smokers and non-smokers (Dallongeville, Marécaux, Fruchart, & Amouyel, 1998) and drinkers and non-drinkers (Tjønneland, Grønbœk, Stripp, & Overvad, 1999) it is important to control for dietary intake.

Despite these major dietary and lifestyle changes, there has been little change in the genetic makeup of humans since the Palaeolithic period about 40,000 years ago and it is possible that the increase in health issues is due to the human genome struggling to keep

up with these changes (Simopoulos, 2002). As in all species, modern day humans have adapted their genes to the environment of their ancestors. In the scheme of evolution, these changes can be seen to be quite sudden, and have accelerated in the last century.

There is evidence to suggest that these rapid changes in diet, along with an increasingly sedentary lifestyle, are promoting the occurrence of chronic health problems such as hypertension, cardiovascular disease, diabetes and some cancers (Simopoulos, 2002). Almost 50% of Americans deaths can be attributed to cardiovascular related illness annually (Sidhu, 2003). These increases in disease and health related problems could be the result of the shift in lifestyle and diet that have occurred over the last 10,000 years.

2.4 Functions of EFAs

N-3 PUFAs such as EPA and DHA are involved in many functions at all levels of performance in the human body, beginning from the cellular level. Every single cell in the human body contains omega-3 PUFAs and the body is reliant on receiving adequate amounts of these acids through dietary sources. Following on from these two points, it can be seen that the type and quantity of omega-3 fatty acids consumed in the diet can impact cellular mechanisms (Yehuda, Rabinovitz, & Mostofsky, 1999). As all brain cell membranes contain n-3 PUFAs, their influence on brain function and behaviour can be seen. Key functions of n-3 PUFAs include eicosanoid production (Larsson, Kumlin, Ingelman-Sundberg, & Wolk, 2004), gene expression (Kitajka et al., 2002) and fluidity of membranes (Yehuda et al., 1999), although at the present time, the exact biochemistry of these processes is not fully understood.

Inflammation

Inflammation forms part of the body's response to infection or injury, and is also part of the cell response within the human body (Feller, Gawrisch, & MacKerell Jr, 2002). Many disorders are characterised by inflammation, including neurodegenerative disorders such as Alzheimer's disease (Galasko & Montine, 2010), cardiovascular diseases (Hansson, 2005)and psychological disorders(Raison, Capuron, & Miller, 2006). The inflammatory process is triggered by a real or perceived threat to tissue homeostasis. It is designed to eliminate harmful pathogens, protect healthy tissue from damage, and initiate repair to damaged tissue. This protective inflammatory response is caused by the up regulation of the transcription of genes which produce inflammatory substances, leading to the release of inflammatory mediators including eicosanoids, cytokines and nitrogen. However, if inflammation is not controlled by the human body, disease or damage to tissue can result.

Evidence has shown that at sufficiently high levels, long chain omega-3 PUFAs have been associated with lower levels of inflammation and can be seen as potentially potent anti-inflammatory agents (Calder, 2006). The effect of long chain omega-3 PUFAs can be both direct and indirect. A direct example is when n-3 PUFAs replace AA as an eicosanoid substrate due to the competitive nature of these acids (Calder, 2006). This then inhibits the metabolism of AA, thus reducing inflammation levels. An indirect example is when long chain omega-3 PUFAs change the expression of inflammatory genes through effects of transcription factors (Calder, 2006). As a result of the anti-inflammatory effects of these acids, it has been suggested they may have some therapeutic potential in diseases associated with inflammation, including cardiovascular disease (Schwalfenberg, 2006).

One possible mechanism of action for inflammation is through the release of inflammatory mediators such as eicosanoids (Calder, 2006). Eicosanoids are short lived, hormone-like lipids with a chain length of 20 carbon atoms, which are generally derived from either omega-3 or omega-6 fatty acids (Larsson et al., 2004; Rosenthal & Glew, 2009). Eicosanoids are formed when PUFAs are separated from membrane phospholipids, and undergo elongation and desaturation processes to become messenger molecules. There are many different types of eicosanoids, with two types of eicosanoids being n3-derived eicosanoids and the n6-derived eicosanoids. As a general rule, eicosanoids derived from n-6 fatty acids have inflammatory effects, while eicosanoids derived from n-3 fatty acids have anti-inflammatory effects. Inflammatory eicosanoids derived from AA are more potent than those derived from EPA (Rosenthal & Glew, 2009; Schmitz & Ecker, 2008). While EPA is another physiologically significant precursor fatty acid for eicosanoids (Rosenthal & Glew, 2009), these eicosanoids are less numerous. This is because EPA comprises less than 1% of the total brain fatty acid composition, despite being incorporated into the membrane of all mammalian cells (De La Presa Owens & Innis, 1999).

In humans, stress initiates inflammatory responses, whether that stress is physical or psychological. As mentioned above, eicosanoids regulate the inflammatory response in the body, with some being pro-inflammatory and others being anti-inflammatory. There is evidence to suggest that adequate levels of EPA and DHA decrease the production of pro-inflammatory eicosanoids by displacing AA from cell membranes(Strokin, Sergeeva, & Reiser, 2004). This indicates a possible link between levels of omega-3 PUFAs and inflammation.

Despite these findings, there are some contradictory research findings. One study used a placebo controlled double blinded design to investigate whether inflammatory markers

in middle aged adults could be affected by n-3 enriched food (Fujioka et al., 2006). One group were given an EPA-rich drink, and the other group were given a placebo. Interestingly, while EPA concentrations in the blood did rise in the experimental group, the inflammatory markers did not significantly change in either group. More trials are needed to investigate these conflicting results.

As there is a competitive relationship between n-3 and n-6 PUFAs, both the amount of n-3 fatty acids and the amount of n-6 fatty acids are relevant. N-3 and n-6 PUFAs compete for the same position on the molecule and require the same enzymes for desaturation and elongation (Burdge & Calder, 2005). As a result of this competition, a sufficiently high dietary intake of long chain omega-3 PUFAS leads to increased incorporation of the n-3 molecules into the membrane phospholipid in the place of the AA-derived eicosanoids (Calder, 2006). This in turn leads to production of higher levels of anti-inflammatory EPA-derived molecules in place of pro-inflammatory AA-derived eicosanoids (Gibney & Hunter, 1993).

As well as reducing inflammation, eicosanoids from dietary n-3 PUFAs have been found to have many different health benefits. While n-3 PUFAs have been found to be anti-inflammatory (Calder, 2006), cardioprotective (Das, 2000) and anti-carcinogenic (Larsson et al., 2004), the mechanisms of these relationships are still poorly understood. This is partially because it is currently unknown to what extent these benefits are directly due to the higher levels of n-3s, and to what extent they are due to the partial replacement of eicosanoids derived from n-6 (Rosenthal & Glew, 2009). It is important to note that the higher the intake of n-3s obtained through dietary sources, the higher the level of their incorporation. As n-3 and n-6 PUFAs have a competitive relationship for desaturation and elongation, the higher incorporation of n-3 PUFAs into the phospholipids, the lower the level of AA derived eicosanoids present. This helps to lower the incidence of conditions that result from an inflammatory state.

Gene Expression

Gene expression, where the products of genes are affected by the genetic information of the gene itself, controls protein production in the cell along with production of other nutrients. Gene expression allows the cell to control the production of different types of proteins such as enzymes, receptors and structural proteins as they are required in the cell by switching genes on and off as required by the organism (Hawkins, 1991). Much research has focused on the effects of n-3 PUFAs on gene expression, as it indicates a direct route to how fatty acids can have an effect on gene function (Jump, 2002). The effects of n-3 PUFAs on gene expression can be rapidly observed, with changes in enzymes

being detected in some cases within hours of animals being fed n-3 enhanced diets (Jump, Clarke, MacDougald, & Thelen, 1993; Jump, Clarke, Thelen, & Liimatta, 1994).

This has been evidenced in animal studies examining the effects of n-3 PUFA supplementation where changes in gene expression have been observed in animals through the use of dietary supplements (Kitajka et al., 2002). The expression levels of certain genes involved in various processes such as energy metabolism and regulatory proteins were modulated to a greater extent in rats fed a diet of high EPA and high DHA chow when they were compared with a control group of rats fed a diet rich with LA (Kitajka et al., 2002). In a follow-up study by the same research group, it was further found that the different expression of the genes was influenced by the LA/ALA ratio, indicating that this ratio may also play a part in gene expression (Barceló-Coblijn et al., 2003). This indicates that the levels of n-3 and n-6 PUFAs in rats can have an impact on cellular function at a very basic level. Based on their findings, the authors hypothesised that this altered gene expression may relate to the link between diet and improvement in cognitive performance, but more research is needed to support this theory.

Membrane Fluidity

All cell membranes throughout the body have similarities in function, despite their diversity. They all separate cells from the external environment and provide a relatively stable internal environment (Liljas, 2009). Additionally, all cell membranes contain active enzymes, ions and transporter molecules, which aid with the function of a cell (Youdim, Martin, & Joseph, 2000). The membrane barrier controls the flow of nutrients and other molecules into and out of the cell (Liljas, 2009). A cell membrane is composed primarily of phospholipids, with an internal hydrophobic layer and a double molecular hydrophilic layer surrounding the membrane (Liljas, 2009). In order for cells to be able to interact with each other, the exchange of ions must take place across the cellular membrane. Maximum efficiency in this process has been found when an "optimal" physical state of the membrane occurs, where it is neither too rigid nor too fluid (Yehuda et al., 1999). The structure of the cellular membrane varies according to the fatty acids which are contained within the tail of the phospholipid. Saturated fatty acids are rigid in structure, allowing the phospholipids to sit in close together. When there are some double bonds present on the hydrocarbon chain, as in the case of EFAs, the properties of the fatty acid are changed. As they become less saturated, there are more double bonds present and the molecule can take more forms. As such, the higher the degree of unsaturation, the more flexible the molecule is.

As n-3 PUFAs contain multiple double bonds, they can take many different formations and affect membrane fluidity. The higher the amount of PUFAs contained in the cell membrane, the more fluid the membrane becomes. As such, the lower the level of omega-3 PUFAs in the diet, the more rigid the bilayer is (Yehuda et al., 1999). With 5 double bonds, EPA can adopt multiple different configurations, but the extra double bond in DHA gives it unique abilities, which is perhaps why it has been found to play a prominent role in modulating cell membrane fluidity (Stillwell & Wassall, 2003).

Membrane fluidity has been found to be associated with brain function. Animal studies have shown that mice that were fed an omega-3 rich diet had a higher synaptic membrane fluidity than control mice (Suzuki, Park, Tamura, & Ando, 1998). Additionally, the mice that were fed the high omega-3 diet showed a higher level of ability in a maze-learning task than the control animals on a palm oil diet (Suzuki et al., 1998). This suggests that the higher cognitive abilities of the mice may be due to the high omega-3 levels in the chow, and provide a link between membrane fluidity and brain function in the mice.

A decrease in membrane fluidity lowers the activity of ions on the membrane and also has effects on neurotransmitter activity. As the production of multiple neurotransmitters has been linked to an increase in omega-3 intake (Chalon, 2006), then omega-3 intake could possibly affect neural activity through the neurotransmitters.

In summary, the evidence in the literature suggest that dietary intake of EFAs can have an effect on the structure of a cell membrane, and in turn affect cellular processes. These changes in cellular processes may have an impact on brain function. While many cellular functions are hypothesised to be affected by membrane structure, it is important to realise that the relationship between the physical properties of the cell is not entirely dependent on the number of double bonds (Youdim et al., 2000). With the knowledge that cell structure and fluidity is not constant, changes in fluidity can be investigated alongside other cognitive and functional changes to see if a relationship exists. If such a relationship does exist, and changes in fluidity relate to changes in function, then it needs to be determined whether this relationship exists due to a flow on effect. It is evident that more research is required in this area, first to determine whether a relationship exists, and if so to then explore the nature of the relationship.

Summary of EFA Functions

While many people limit the intake of fat in their diet in order to control their weight, fats are still necessary in the human body. Fats are molecules composed of carbon and hydrogen and are insoluble in water (Widmaier, Raff, & Strang, 2006). They have many

functions, including maintaining a healthy body temperature and providing cushioning against trauma for major organs. Lipids also form a large portion of neural tissue, and also form the main element of cell membranes. Fat is the body's major source of energy and accounts for nearly 80% of the energy stored in the body (Widmaier et al., 2006) . In the past, lipids were viewed primarily as energy sources, however research has established that they also play a key role in many other biological processes such as neurotransmitters, hormones, cognitive and physiological processes (Yehuda et al., 1999).

Despite these important functions of fats in the body, there is a lack of certain types of fats in the typical human diet, especially omega-3 fatty acids (Simopoulos, 2002). These nutrients have been found to be involved with the development of higher order brain functions such as problem solving and memory (Kochman & Czauderna, 2010). Due to this, a chronic deficiency in these fats may have an impact on neurochemical processes, and negatively affect the overall functionality of the human brain.

2.5 Conclusion of chapter

This chapter provided a general overview of EFAs in the human body. It explains how they are lacking in the typical diet of humans, and how this has changed over time. It provided an overview of their structure and functions and also provided an explanation of some of the biological mechanisms which underlie the beneficial effects of EFAs. While it did not fully explain all of the details of the mechanisms, general information was provided to explain how EFAs are obtained, absorbed and then metabolised. It provided an insight into how the consumption of these nutrients has changed over time, and looked at their function. The next chapter will review the literature investigating whether EFA status has an effect on cognitive performance and cardiovascular function, both from an epidemiological and a clinical perspective.

Chapter 3 Effects of essential fatty acids

3.1 Abstract

The purpose of this chapter is to further investigate how essential fatty acids might influence cardiovascular and cognitive performance in the human population. As such, the current chapter will review the scientific literature pertaining to the effect of essential fatty acids in the body, with particular reference to and their effects on cardiovascular function and cognition in adults.

3.2 Effect of EFAs on Cognitive Function

Essential fatty acid status has been linked to cognitive function. This is partly because omega-3 fatty acids have been implicated in reduced learning capacity in regards of memory (M. A. Crawford et al., 2008). Despite the extensive literature on the effect of omega-3 fatty acids on other domains, such as cardiovascular functioning and cellular mechanisms, there is a comparatively small amount of research conducted on the effects of EFA levels on cognition. PUFAs are necessary for the normal growth and development of the brain (Gómez-Pinilla, 2008) and it has been suggested that high concentrations of DHA play an important role in the brain function of all ages, ranging from infants to the elderly (J. Cohen et al., 2005; Fontani et al., 2005; Kidd, 2007; Uauy & Dangour, 2006). DHA is enriched in the brain and is involved in the maintenance of normal neurological function (Su, 2010). As these acids are found in numerous brain cells, it has been suggested that they may play a fundamental role in cognitive function (Lunn & Theobald, 2006). While research has been conducted in this area, mixed results have been found and it is not yet determined whether such a relationship exists.

The literature is particularly limited with respect to young, healthy control subjects. Until now, the majority of research in this area has been targeted at children, elderly and clinical populations. The brain is constantly developing, and is not fully formed until late adolescence or even early adulthood when full reasoning abilities are present (Lunn & Theobald, 2006). In order for the brain to function at its maximum level, the levels of essential fatty acids in the brain must be maintained even after the brain has stopped developing. This validates the need for research in young adults, and it is advantageous to study the brain through all stages of development. By giving researchers an insight into the capabilities of the fully developed brain, research can help to develop reference values for which to compare the effects of cognitive decline.

This section will show the findings of research into the effects of omega-3 fatty acids on cognitive function, with a particular focus on young, healthy subjects. It will begin with animal research and then provide an overview of research in humans.

Animal Studies

Animal research has provided many insights into the effect of dietary n-3 PUFAs on cognitive function, with a large amount of research in EFAs being conducted on animals, primarily rodents. The short breeding time of rodents gives researchers the ability to collect a large amount of data in a relatively short amount of time and their size makes them easy to handle. Additionally, rodents have a level of similarity in brain physiology to humans, making it easier to infer causes of brain diseases. Animal protocols also allow for the control of many extraneous variables that cannot easily be controlled for in human studies, such as dietary intake, exercise levels and compliance with supplementation.

Unlike in human studies, variables that can't be measured without a post-mortem brain analysis can be measured in animals. Parameters that are difficult to measure in humans after an intervention but which can be measured in animals include neurotransmitter levels and neurotropic factors. Additionally, structural alterations in the brain can be measured in animals. It is possible to conduct post-mortem brain analyses on animals which are not possible to do on humans. Many of the imaging techniques that are used on humans can also be conducted on animals, meaning similar data can be collected from humans and animals. For these reasons, many concepts are discovered in rodents and then transferred to humans for additional testing.

Findings of Animal Studies

Animal studies have suggested that DHA is important for brain development and can have an effect on neural function. Wu, Ying, and Gomez-Pinilla (2008) investigated the possibility of a synergistic effect between DHA supplementation and exercise on cognition as well as neural plasticity in rats. As both diet and exercise are parts of daily life, it may be assumed that the effects these variables have on the brain are complementary, in that the benefits they provide combine in a way that enhances their individual benefits (Wu et al., 2008). Wu et al. (2008) conducted a study investigating if there is an interaction between exercise and dietary intake on the central nervous system and whether the combination of exercise and DHA supplementation have a greater effect on cognition than their separate effects.

In order to conduct this research, rats were divided into two groups after one week of standard chow. One group were given a DHA-enriched diet of 1.25% DHA and the other

group were given a diet containing regular chow. They were fed this diet for one week, with or without voluntary exercise. The rats were then again divided into two groups, making four groups in total. These groups were defined as RD-Sed (regular diet, sedentary), RD-Exc (regular diet, exercise), DHA-Sed (supplemented diet, sedentary) and DHA-Exc (supplemented diet, exercise). The animals were evaluated on various tests for their cognitive ability, including the Morris Water Maze test. After being euthanized, the animal's brains were stored for biochemical analyses.

Results showed that a 12-day exposure to a DHA-enriched diet increased learning ability in rats, displayed by improvement in spatial learning in the Morris Water maze test. These effects were found to be enhanced by the combination of exercise and supplementation. The authors suggest that a diet rich in DHA may enhance the effect of exercise on cognition (Wu et al., 2008).

These results have been replicated in different studies. A reduction in brain DHA levels in rats was associated with poor performance in a water maze memory task (Lim, Hoshiba, Moriguchi, & Salem Jr, 2005). Pups were fed either artificial n-3 deficient, artificial n-3 adequate diets or a control diet consisting of mother's milk. Behavioural tasks were completed at 9 weeks of age. The rats who were fed the n-3 deficient diet had poorer memory retention in the water maze test than the rats who had been fed the n-3 adequate diet or the control mother fed rats (Lim et al., 2005).

Overall, the findings of animal studies provide evidence for a beneficial effect of omega-3 supplementation on cognition in animals (Lim et al., 2005; Wu et al., 2008). These findings suggest that omega-3 fatty acids are beneficial in domains such as memory and spatial awareness (Su, 2010). This indicates they affect parts of the brain including the parietal cortex, implicated in spatial processing, and the temporal and frontal cortex, associated with memory (Su, 2010).

Interestingly, there is very little animal research literature that uses EPA as a supplementation. As there is so little research pertaining to the effects of EPA on cognition in healthy animals, the differences between EPA and DHA supplementation on brain function and cognition have not been determined. Future research could compare the two supplementations to help determine whether EPA supplementation leads to similar or different cognitive effects than DHA. More research is needed in this area to determine how this relationship works, and the mechanisms that underly it.

While most research on omega-3 supplementation in animals finds beneficial effects, it is important to consider the limitations of the study design. The supplementation is not

uniform across studies as many of the supplements have varying levels of PUFAs. This can make it difficult to compare results across different studies. For this reason, each study must be taken on its own merits. Future research could compare the amounts of DHA and EPA in each of the supplements being used for supplementation and see if there is a relationship between this and performance.

As this body of research shows, the relationship between cognitive function and omega-3 supplementation is complex, even in rats. There are many factors involved with the relationship, and isolating the effects of particular variables is complex. As this relationship has been shown to be complex within an animal population, it can only be assumed that the level of complexity increases when it is within a human population. With all the additional variability in the existence of a human being than a laboratory raised rat, and the potential benefits to society of improved cognitive function, it can be seen that more research into the area of cognition and omega-3 supplementation is justified.

Human Studies

The animal studies outlined above suggest that there is a relationship between cognitive function and EFA levels. While this is useful information, and a good scientific basis for further research, it is necessary to further test this in human subjects. In comparison, human studies can provide a wealth of information on complex cognitive mechanisms such as language, memory and attention, which cannot be easily studied in animals. Nutritional intervention studies are usually utilised to collect human data, and the double-blind randomised control trial is commonly used. This data collection method randomly allocates participants to a supplementation group where both the researchers and the participants are blinded to supplementation group membership. Additionally, these are generally placebo-controlled. Alternative study methods include epidemiological studies and correlational studies.

There are many observational studies suggesting that poor omega-3 levels are associated with poor cognitive performance in middle aged to older populations (Conquer, Tierney, Zecevic, Bettger, & Fisher, 2000; Nurk et al., 2007). The validity of these studies can be questioned however, due to the limited reliability of self-report food recall diaries and food frequency questionnaires, and this is why clinical trials are required alongside the observational studies. Despite the large amounts of observational research, it is more difficult to determine whether supplementation of omega-3 can improve cognition, due to conflicting results arising from clinical trials.

Epidemiological Research

Alongside the wealth of evidence from animal research there is also epidemiological evidence to support a relationship between fish consumption and better cognitive performance. These studies have been conducted in participants in different stages throughout their lifespan. This helps researchers to determine whether omega-3s can have a beneficial effect in people with or without age-related cognitive decline.

If omega-3 intake can be shown to affect cognition in an adolescent population, it indicates that omega-3 EFAs influence cognition in healthy brains as well as ageing brains or brains from a clinical population. This is because adolescent brains are far from age-related cognitive decline. A prospective epidemiological study conducted in Sweden found that integrating regular fish meals into the diet was associated with better cognitive performance in adolescents irrespective of socio-economic status (Åberg et al., 2009). With a sample size of over 3000 participants, responses on a questionnaire about fish consumption undertaken at 15 years of age were correlated with higher levels of intelligence when tested at age 18 (Åberg et al., 2009).

In a longitudinal, population based study conducted on 1,475 adults aged above 55 years, daily n-3 PUFA supplements were significantly associated with lower risk of cognitive decline over a median period of 18 months (Gao, Niti, Feng, Yap, & Ng, 2011). Cognitive decline was defined as being at least a two point drop in MMSE score from baseline to follow-up. These findings were significant after controlling for variables such as age, gender, the presence of vascular risk, nutritional status as well as many other variables (Gao et al., 2011). It must be noted that this study used a survey based method and did not involve blood tests to verify responses. Additionally, these findings were based upon supplement usage, and did not initially include data pertaining to dietary intake.

Babies and Children

The cognitive benefits of essential fatty acids can be gained from very early in life, with research showing that maternal consumption of DHA during pregnancy can increase a child's IQ later in life (J. Cohen et al., 2005). These results must be interpreted with caution however, as the levels of DHA consumed by the mothers were far higher than what would be expected from normal levels of fish consumption (J. Cohen et al., 2005). In order to address this issue, results need to be looked at on a population level and not as individual results.

Essential fatty acids have also been used in a clinical setting with children suffering from developmental coordination disorder (Richardson & Montgomery, 2005). In a study

known as the Oxford-Durham trial (Richardson & Montgomery, 2005), researchers conducted a randomised controlled trial of n-3 and n-6 essential fatty acids with 117 subjects. The children were treated for 3 months with either a high EPA/DHA supplementation or a placebo, then the same children received the opposite condition. Results showed an improvement in reading and spelling in the active treatment group versus the placebo group in the first part of the crossover. Children who had received the active treatment first maintained their progress over their placebo treatment, whereas the placebo-treatment group had similar improvements after the crossover (Richardson & Montgomery, 2005). This suggests fatty acid supplementation can improve cognitive processes in children via supplementation (Richardson & Montgomery, 2005).

Healthy Adults

Surprisingly, there have been few studies conducted on a healthy young adult population investigating the cognitive benefits of n-3 supplementation. Of these studies, there are inconsistencies in the findings which may be due to factors such as the methodological and environmental complexities in human research.

In one study investigating the effect of omega-3 supplementation on cognition in healthy subjects (Fontani et al., 2005), omega-3 was administered to participants with no clinical signs of cognitive decline, aged between 22 and 51 years. Participants were given 4g of fish oil daily, consisting of 1.6g of EPA and 0.8g of DHA for 35 days. Concurrently, a control group received olive oil as a placebo. As expected, the omega-3 supplementation reduced the AA/EPA ratio. After receiving the supplementation, significant decreases in anxiety, fatigue and depression scores were observed. There were also improvements in cognitive functioning found, demonstrated by a decrease in reaction time in sustained attention tasks and go/no-go tasks, and a decrease in the number of errors (Fontani et al., 2005). It must be noted however, that in this study, all participants completed at least four hours exercise a week as part of the inclusion criteria. This should be considered when interpreting the results, as it is possible that the relatively high levels of physical activity may have acted as a mediating variable on the cognitive performance of the participants. This is supported by the previous animal research discussed above, which suggest that a diet rich in DHA may enhance the effect of exercise on cognition more than either diet or exercise alone (Wu et al., 2008).

In conflict with these findings, research conducted by De Groot and colleagues found that AA, EPA and DHA were not significantly associated with cognitive performance either at baseline or after the 22 week supplementation period (de Groot, Hornstra, & Jolles, 2007). In support of these findings, results from a study conducted on a college aged sample

replicated these results, with results showing no benefit of omega-3 supplementation over a four week period (Karr, Grindstaff, & Alexander, 2012). It must be noted that it was acknowledged by the authors that the sample size was small and it is possible that the supplementation dosage was below therapeutic levels (Karr et al., 2012).

Older Adults

A trial examined the associations between fish consumption, the intake of EPA and DHA from fish and other foods, and cognitive decline over 5 years (Van Gelder, Tijhuis, Kalmijn, & Kromhout, 2007). As fish is such a rich source of essential fatty acids, this indirectly looks at the relationship between essential fatty acids and cognitive decline. With a sample of 210 healthy participants, aged between 70-89, the researchers collected cognitive data five years apart. Fish consumption was also recorded over the five-year gap between data collection. A significant negative linear trend was detected between fish consumption and cognitive decline. This research suggests that a moderate level of n-3 acid can slow the rate of cognitive decline (Van Gelder et al., 2007).

In spite of the large amount of literature supporting an effect of essential fatty acids on cognitive function, there is still conflicting evidence on this topic. Many studies suggest that essential fatty acids do have an effect on cognitive performance (J. Cohen et al., 2005; Fontani et al., 2005; Kidd, 2007; Uauy & Dangour, 2006) however other studies have found no such evidence (Antypa, Van Der Does, Smelt, & Rogers, 2009; de Groot et al., 2007; Rogers et al., 2008). A trial conducted by Dangour in 2010 investigated whether n-3 long chain PUFA supplementation would affect cognitive function in cognitively healthy older people (Dangour et al., 2010). Over a supplementation period of 24 months, no difference in cognitive decline was detected by the researchers. While this study made some important findings, there were some methodological issues with it that may have affected the outcomes of the study. The dosage of PUFAs in the supplements only totalled 700mg daily. This is a relatively low dose of omega-3 in a supplementation trial, and results may have differed if a higher dosage was used in the trial. Additionally, the study did not exclude participants who had a high intake of fish habitually. As these people already had a high intake of omega-3, the low dosage of supplementation may not be enough to elicit cognitive benefits.

Although Assisi and colleagues (2008) suggest that reduced levels of n-3 long-chain polyunsaturated fatty acids are associated with mental disorders of the central nervous system, his research failed to find any significant effects of supplementation in these patients. This lack of results may be due to the clinical nature of his population, and is one

reason why a clinical sample is not optimal. Another possible reason for this lack of support is that 12 weeks of supplementation may not be long enough to see a change.

All results must be also be interpreted with caution as the type and dosage of supplementation ranged greatly across the different studies. This data suggests that a relationship may exist, but further research is still needed to investigate the extent of this relationship, and what the relationship means. It is difficult to compare results between studies due to the large amount of heterogeneity in study design, supplementation types and dosages, sample demographics and length of intervention. A way to rectify this is to measure participants at a baseline level, therefore seeing their true level of essential fatty acids. By eliminating the issue of duration of supplementation, a clearer idea of the effect of essential fatty acid status on cognitive performance may be obtained.

While there is evidence indicating benefits of essential fatty acids on cognition, there is still much more research to be conducted. Based on this preliminary evidence, there is a need for further research in this area, in order to further understand the relationship between omega-3s and cognition. Alternatively, controlled clinical trials could be conducted with specific hypotheses based on the omega-3 dosage used. These studies could provide further evidence of effects of essential fatty acids on brain function.

3.3 Effect of EFAs on Cardiovascular Health

As well as its links with cognitive performance, essential fatty acid status has also been linked to cardiovascular performance. Coronary disease presents a major problem to Western society, with almost 50% of Americans deaths related to cardiovascular related illness annually (Sidhu, 2003). PUFAs have been found to have an impact on the prevention and treatment of coronary heart disease (Wijendran, 2004). Consequently, a better understanding of the relationship between the cardiovascular system and PUFAs may continue to reduce the risk of future cardiovascular events and lower the broad effect of such diseases within the community. The question will be addressed at a range of levels, including epidemiological studies, laboratory studies and randomised controlled clinical trials.

Epidemiological Research

The epidemiological research approach focuses on investigating dietary intake on a population level. By looking at the cardiovascular health of a population of which fish forms a staple part of the diet, researchers can investigate how fish intake affects health. Due to the high levels of essential fatty acids present in fish, a population that habitually consumes large amounts of fish can be expected to have a higher and more balanced

essential fatty status that the population who eats almost no fish. These methods allow for the observation of nutrients consumed by people across their lifespan, ranging from infants to the elderly.

Evidence for the theory that higher levels of PUFAs are beneficial for cardiovascular health has been provided through studies looking at fish consumption within different cultures and the occurrence of cardiovascular problems within that population (Bang et al., 1980). Research suggests the Inuit people have a lower mortality rate from coronary disease than that of the western world and has been related to their high fish consumption (Bjerregaard & Dyerberg, 1988). As the dietary intake of these people contains large amounts of EFAs, and they have a lower chance of heart disease, it has been suggested that long chain PUFAs may help to prevent cardiovascular disease.

Research began in this area over 30 years ago, when it was found that people living in Greenland had a low rate of ischemic heart disease (Bang et al., 1980). Seal and fish are a predominant feature in the diet of this population, creating a diet rich in essential fatty acids. Bang et al. (1980) compared a sample of Inuit participants with a control group of Danish participants, whose diet contained significantly less fish. Blood tests showed that as well as the significant differences in the total amounts of essential fatty acids, differences were also detected in the types of EFAs present. The Inuit population had higher levels of the n-3 linolenic fatty acids and the Danish participants had more of the n-6 linoleic fatty acids. Additionally, there was a significant difference in the ratio of the n-3 to n-6 fatty acids between the two groups. The Inuit population who had a healthier n-3 to n-6 ratio, also had a significantly lower average level of heart disease than the control population (Bang et al., 1980).

In a study of 1800 participants conducted over a 25 year period, researchers detected a difference in the pattern of disease when comparing a Greenland population with populations from other Western European countries (Kromann & Green, 1980). Participants were recruited from a district populated mainly by whalers and sealers, and hence had a very high average intake of fish. The occurrence of many diseases was investigated, including cardiovascular events, cancer and diabetes mellitus. In the time period studied, rates of acute myocardial infarction were lower than expected (Kromann & Green, 1980). Since this early work, these findings have been replicated in a Japanese population, where an association between higher fish intake and lower cardiovascular risk was found through higher n-3 PUFA consumption and habitual low fat diet (Mizushima, 1997).

The relationship between fish consumption and lower risk of cardiovascular disease is not limited to the Inuit and Japanese population, but has also been documented in western society through longitudinal research. Daviglus (1997) found significant associations between baseline fish consumption and the 30-year risk of death from non-sudden myocardial infarction . A sample of 1822 men was tested in 1957, and was followed up 30 years later (Daviglus, 1997). Detailed dietary information was collected, including typical eating patterns and what quantities of certain food the men ate, including fish. Results showed that eating fish was beneficial for general health. With the exception of carbohydrates, saturated fatty acids and monounsaturated fatty acids, all of the beneficial nutritional variables measured were highest in the men with the largest amount of fish consumption (Daviglus, 1997).

During 30 years of follow-up, there were 1042 deaths in total among the group. Of these 1042, 573 were from cardiovascular disease, 430 of these from coronary heart disease, and 293 of those from heart attack (Daviglus, 1997). A trend in the data was detected, where lower mortality rates from cardiovascular problems were significantly correlated with higher fish consumption. Men who consumed 35g or more of fish per day had a 42% lower rate of death from myocardial infarction, compared to men who ate no fish. No significant relationship was found between fish consumption and any other type of cardiovascular disease other than non-sudden myocardial infarction. While this study does provide some evidence for the benefits of fish oil regarding cardiovascular disease, conclusions from this study are limited due to the narrow range of significant results.

Further evidence supporting these findings indicate a significant difference in the percentage of deaths caused by cardiovascular disease between Western countries (45%) and the population with a higher fish intake (7%) (Simopoulos, 2008). An ecological study involving 36 countries found that fish consumption was significantly associated with a decreased risk of ischemic heart disease in both sexes (Zhang, Sasaki, Amano, & Kesteloot, 1999). This relationship was consistent when the consumption data was collected 2, 12 or 30 years prior to the death (Zhang et al., 1999).

Alongside the extensive range of research supporting the benefits of fish oil in cardiovascular disease, some research has found no beneficial effect of fish oil on cardiovascular health in specific areas (Guallar, Hennekens, Sacks, Willett, & Stampfer, 1995). Guallar et al (1995) conducted a study with 14,916 participants. Despite the hypothesis predicting a beneficial effect of high fish oil levels in the blood in relation to myocardial infarction, results showed no link between the incidence of a first heart attack and fish oil intake.

There are many reasons why there is conflicting epidemiological data. Some literature suggests that the conflicting information from epidemiological data may be due to only a small fraction of the sample population reporting low or no fish intake (Albert et al., 1998), as inverse associations between fish consumption and coronary mortality have been detected mainly in populations including large proportions of low fish eaters (Kris-Etherton, Harris, & Appel, 2002). This suggests that cardio-protective properties of fish oil may only be present in diets that lack adequate levels of fish.

While the lack of cohesion in results may suggest the need for more research in the specific area of myocardial infarction and essential fatty acid status, a large amount of epidemiological research suggests that certain essential fatty acids may provide a protective mechanism against heart disease, possibly suggesting that higher levels of EFA intake may lead to a lower risk of cardiovascular disease. The above information indicates that humans need adequate levels of n3 and n6 in their diet for their cardiovascular system to function optimally.

Clinical Research

Much clinical research in the area of n-3 fatty acids and cardiovascular disease has been conducted, and there is a large variance in the research conducted. Studies vary in size, quality and methods, and this has led to mixed results and some confusion of the relationship between variables. While some evidence points to the benefits of omega-3 supplementation, other research has found no such evidence. More clinical trial research is needed to gain a better understanding of the effect of omega-3 supplementation, and even more so in the under-represented, younger and healthy population.

A review of randomised clinical trials investigated the effect of dietary and supplemental intake of n-3 fatty acids on certain cardiovascular parameters (Bucher et al., 2002). It included eleven different trials, based on strict inclusion criteria. Consisting of 7951 patients in the intervention group and 7855 in the control groups, the analysis suggested that a diet supplemented with omega-3s decreased mortality due to myocardial infarction, sudden death and overall mortality in patients with coronary heart disease (Bucher et al., 2002).

The GISSI trial investigated whether n-3 PUFAs can influence morbidity and mortality in a population of patients who are suffering from differing types of heart failure. A multi-centre, open-label omega-3 and placebo controlled trial was conducted on 11,324 participants at 172 different sites (Marchioli, 1999). It investigated the effect of omega-3 supplements on participants who had recently suffered a myocardial infarction.

Treatments were highly purified EPA and DHA at a dosage of 1000mg daily. The study found that treatment with omega-3 PUFAs led to a clinically important and statistically significant effect of a lower mortality rate in patients when compared with controls (Marchioli, 1999). It must be noted however, that this was not a blinded trial so results gained from it must be viewed conservatively.

Most of the trials described above were conducted on samples with pre-existing health conditions (Bucher et al., 2002; Marchioli, 1999), and in the older age bracket. This may be seen to only investigate whether n-3 PUFAs can have an effect on people who have pre-existing cardiovascular issues. Further research is required in this area in order to provide a clear picture of the relationship between cardiovascular performance and omega-3 fatty acids. This research could include, but is not limited to, further randomised clinical trials to determine whether omega-3 decreases the risk of cardiovascular events and additional studies to determine whether this effect is due to EPA, DHA, the ratio between them, or a combination of these factors.

Secondary Prevention Research

As well as clinical and epidemiological research, there is also a large body of research looking at the effect of diet as secondary prevention for coronary events and death. The Lyon Diet Heart study (De Lorgeril et al., 1994), was conducted on over 600 participants over 5 years. The aim of the study was to reduce cardiovascular mortality after a myocardial infarction through the use of diet. Participants were allocated to one of two diet groups; either a Mediterranean style diet that was high in alpha-linolenic acid, a precursor of n-3 long chain fatty acids or a control diet. The Mediterranean diet was chosen because it incorporates the concepts of healthy eating compared to a typical diet by including more fish, less meat, more vegetables and replacing butter and margarine with a spread provided by the study. Over a 5 year period with yearly visits, there were 33 cardiovascular events in the control group and eight in the experimental groups, with this difference being statistically significant (De Lorgeril et al., 1994). The results of this trial found that the Mediterranean diet rich in alpha-linolenic acids was more efficient in the prevention of coronary events and death when compared with a control diet, suggesting that diet can play a role in the secondary prevention of cardiovascular events. If these results have been found in dietary studies, then it could be hypothesised that supplements high in EPA and DHA could have similar effects.

Arterial Stiffness

Omega-3 fatty acids have been shown to have an impact on the prevention and treatment of coronary heart disease, both clinically and epidemiologically (Bang et al., 1980; Bucher

et al., 2002; Wijendran, 2004). As arterial stiffness is a potential risk factor for cardiovascular disorders (Australian Bureau of Statistics, 2006; Vlachopoulos, Aznaouridis, & Stefanadis, 2010), the relationship between arterial stiffness and essential fatty acids could be an important factor in the complex relationship between cardiovascular disease and omega-3 essential fatty acids. However, the relationship between arterial stiffness and EFAs is not fully understood, particularly in a younger population.

In order to understand arterial blood flow, it is important to have an understanding of how the cardiovascular system works. Throughout the lifespan, the heart acts as a pump, with its primary purpose being to deliver blood around the body. This is a continuous process and occurs every minute of every day. The heart beats approximately 100,000 times a day pumping approximately 8000 litres of blood (Martini et al., 2008). Over time, this process puts the heart under stress and research has shown that arterial function changes with increased age (O'Rourke, 2007). Arterial blood flow begins in the left ventricle of the heart. When this ventricle contracts, the blood is then pumped into the aorta and travels through the arteries to the target organs. This period is known as the systole. At the end of the systole, the valve to the aorta closes and the mitral valve opens. The diastole, the time between heart beats, then begins and the heart refills with blood through the mitral valve in readiness for the next systole phase (M. N. Levy & Pappano, 2007).

Arterial stiffness relates to the elasticity of the arterial walls, and their ability to adjust to pressure changes. The arterial wall is composed of multiple layers, and the structure of the arterial wall differs based on the arteries function and location (M. N. Levy & Pappano, 2007). The endothelium is the innermost layer and is composed of a single layer of cells. These cells are surrounded by a layer of elastic fibres known as the elastic lamina. This in turn is surrounded by smooth muscle cells and the outermost layer, known as the adventitia, is made up of connective tissue made of collagen fibres. When the endothelial cells are stimulated, this elicits a response in the smooth muscle cells with the greater the contractile activity of the smooth muscle of an arteriole, the smaller its diameter (M. N. Levy & Pappano, 2007). Stiffer arteries have a reduced capacity to expand and contract in reaction to pressure changes (Cecelja & Chowienczyk, 2012).

Stiffness of arteries can be a result of many factors, including age (M. N. Levy & Pappano, 2007; O'Rourke, 2007), genetics (Lacolley, Challande, Osborne-Pellegrin, & Regnault, 2009) and lifestyle factors (Vlachopoulos, Alexopoulos, & Stefanadis, 2007). Throughout the lifespan, large arteries tend to stiffen as a result of two primary factors, loss of elastin

and the accumulation of collagen (Tanaka & Safar, 2005). When elastin is present in arterial walls, the walls are able to adjust in diameter in response to changes in blood pressure. As the level of elastin lowers, a gradual process that begins between the ages of 30 and 80 (Lakatta, Mitchell, Pomerance, & Rowe, 1987), and the arteries are unable to expand to the same extent narrowing the artery and restricting the blood flow. This degeneration of fibres is correlated with an increase in collagen production (Laurent, Boutouyrie, & Lacolley, 2005), which further restricts the capability of the arteries to expand.

One way to independently measure arterial stiffness is through pulse wave velocity (PWV) and pulse pressure measurements. Pulse pressure is the difference between the systolic and the diastolic pressure readings and is a representation of the force generated by the heart each time it contracts (Benetos et al., 1997). PWV is the rate which this pulse pressure wave travels down the arteries. It is calculated by dividing the distance travelled by the wave by the time it takes the wave to travel this distance. This technique works by measuring the pulse times at two sites in the arterial tree, with higher level of stiffness indicated by a higher PWV (Mattace-Raso et al., 2006). This technique is commonly used, as it is non-invasive and produces reliable and repeatable results (Vappou, Luo, & Konofagou, 2010). The speed of the wave will be faster in a stiffer artery, as a stiff artery cannot adjust to pressure changes as the blood flows through it, with the presence of such adjustments slowing the velocity of the wave (M. N. Levy & Pappano, 2007).

Arterial stiffness has been associated with a large number of risk factors for cardiovascular disease (Laurent et al., 2006). A large meta-analysis, comprising 17 longitudinal studies with 15,877 participants, found that aortic pulse wave velocity was a strong predictor of the relative risk of cardiovascular events, mortality and all-cause mortality (Vlachopoulos et al., 2010).

In addition to its relationship with cardiovascular outcomes, arterial stiffness has also been associated with cognition. Relationships have been detected between cardiovascular performance and cognition through arterial stiffness, with certain cognitive domains such as working memory declining with elevated arterial stiffness (Waldstein et al., 2008). A systematic review found that increased levels of arterial stiffness significantly predicted a longitudinal decline in performance on the MMSE (Pase, Herbert, Grima, Pipingas, & O'Rourke, 2012). This was supported by the findings of another meta-analysis with results suggesting that higher PWV is a significant predictor of future cognitive decline (Rabkin, 2012).

However, these findings are not conclusive, as contradictory evidence has also been found. The findings of a prospective population-based study did not identify arterial stiffness as an independent risk factor for cognitive decline (Poels et al., 2007). This is surprising, as the same study found that higher levels of aortic stiffness predicted poorer cognitive function in a cross-sectional fashion. These conflicting results may be because those who participated in the longitudinal follow-up study were younger and had lower levels of arterial stiffness than those who did not continue (Poels et al., 2007). The authors acknowledge that as age and cardiovascular factors are associated with cognitive function, this selective attrition may have limited the ability to find an association between cognitive decline and arterial stiffness (Poels et al., 2007). These opposing research findings used different methodologies, possibly leading to these contradictory results. As such, further research is required to clarify relationships between cognitive domains and arterial stiffening. Any associations should be viewed with caution, until results are validated by multiple sources.

It must be noted that, similar to other areas examined in this thesis, the majority of literature in the field focuses on older samples, generally neglecting those less than 50 years of age. Although arterial stiffening has been related to cognition across the life span, the existence of this relationship for a population less than 50 years of age is uncertain. Through researching cognitive performance in relation to arterial stiffening in a younger population, cognitive performance may be viewed as a variable not specifically associated with older cohorts, and will therefore emphasise the necessity for further research in this age group. Research suggests that arterial function peaks around 17 to 30 years of age, followed by a gradual decline with a more steep decline after 60 years of age (O'Rourke, 2007). As arterial function is considered to gradually decline from about 30 years of age (O'Rouke, 2007), its impact on cognition may also begin at this age. However, it is possible that cognition is not affected by lower levels of arterial stiffness associated with younger groups and therefore, cognitive decline may be undetectable. Hence, further investigation is required to clarify the existence of this relationship in a younger cohort. Through conducting research on a cohort within the age range of that peak, researchers can examine the effects of EFA status on arterial function, without the extraneous variable of age-related decline.

Relationship with Omega-3

In relevance to this thesis, current research suggests that long chain omega-3 fatty acids may be beneficial in reducing arterial stiffness (Pase, Grima, & Sarris, 2011). Using data pooled from 10 independent studies, the authors found that omega-3 fatty acids were

effective in improving pulse wave velocity and arterial compliance with small to moderate clinical effects (Pase et al., 2011). The authors suggested that reduction in arterial stiffness may account for some of the cardio-protective effects of omega-3, and that further research could determine the optimal dosages of EPA and DHA required to reduce arterial stiffness (Pase et al., 2011).

Cerebral blood flow

Research has suggested an underlying link between brain function and the cardiovascular system, with increased cognitive performance being related to adequate blood flow to the head (Kandel, Schwartz, & Jessell, 2000). The brain is dependent on a constant blood flow to receive the oxygen required for cognitive processes. This is most apparent in extreme cases, such as haemorrhage or stroke, but vascular health can also deteriorate over many years (Franklin et al., 1997) resulting in much more subtle signs of damage. The brain requires a constant supply of blood in order to maintain cellular integrity and for information processing (Buunk, Van Der Hoeven, & Meinders, 2000; Sinn & Howe, 2008). This constant blood flow provides the brain with its energy requirements, primarily oxygen and glucose, to be delivered into cerebral tissue. These nutrients need to be delivered to the brain via the blood, and absorbed through the blood brain barrier. While glucose is the principal energy source for the brain, the brains ability to store it is extremely limited. Hence the brain is reliant on a constant supply of blood to provide glucose. As a result of this, it can be seen that cerebral blood flow plays a vital role in brain function, due to its role in transporting nutrients across the blood brain barrier.

This is further demonstrated by the observation that despite only accounting for 2% of bodyweight, the brain consumes 15% of the cardiac output, demonstrating the high oxygen requirements of the brain (Kandel et al., 2000). This adequate blood flow and the oxygen levels necessary for cognition is strongly associated with a healthy cardiovascular system, thus strengthening the link between the cardiovascular system and cognitive output.

Additionally, hypo-perfusion has been linked to many diseases and conditions, including Alzheimer's disease (Suo et al., 1998) and stroke (O. Y. Bang et al., 2008). Specifically, a longitudinal study of 582 participants demonstrated that as participants aged , those with a higher PWV at baseline showed a decrease in performance on certain cognitive tasks (Waldstein et al., 2008). These tasks included verbal and non-verbal learning and memory and a cognitive screening measure weighted for memory and concentration (Waldstein et al., 2008). As discussed earlier in this chapter, arterial stiffness, a sign of cardiovascular disease, has been linked to cognitive decline (Pase et al., 2012; Waldstein et al., 2008).

Pathological and non-pathological levels of arterial stiffness caused by loss of elastin and increased collagen in arterial walls, has been demonstrated as an independent predictor of cognitive decline and Alzheimer's disease (Hanon et al., 2005). If blood flow velocity is associated with these diseases, then preventing low blood flow could help prevent these diseases. There is also research suggesting a connection between vascular risk factors and cognitive decline (Zlokovic, 2008), but as yet this connection remains unclear. It has been theorised however that increased blood flow to the brain could potentially work as a protective mechanism against cognitive decline (Zlokovic, 2008).

Roman et al. (1997) demonstrated that after cardiac surgery, certain cognitive abilities improved, such as memory. The author hypothesised that this could be due to the increased blood flow to the brain that occurred after the surgery. The occurrence of people waiting for heart transplants underperforming on cognitive tasks has been demonstrated by other research in which heart transplant candidates with end stage cardiac disease performed significantly worse than controls on tasks measuring psychomotor speed and mental flexibility (Putzke et al., 2000). Another previous study, containing only heart transplant candidates, found that pulmonary artery pressure and right atrial pressure were significantly related to decreased performance on cognitive tasks (Putzke, Williams, Rayburn, Kirklin, & Boll, 1998). This research suggests that cognitive performance is affected by cardiovascular health, providing a possible mechanism by which cardiovascular health may be associated with cognitive function. It must be taken into account however that other factors may also play a part, including stress, age and general health. One proposed mediation model of physical activity, the cardiovascular system and cognition suggested that physical activity enhances cardiovascular health thereby increasing cognitive performance (Eggermont, Milberg, Lipsitz, Scherder, & Leveille, 2009). This shows there is a growing appreciation within the scientific community of factors, such as cardiovascular health, that may indirectly affect cognition (Deary et al., 2009).

This has also been supported by meta-analysis research. A meta-analysis study of transcranial Doppler research found that participants with AD and vascular dementia had reduced cerebral blood (CBF) velocity when compared with healthy age-matched control participants (Sabayan et al., 2012). The Rotterdam study also found that people with higher CBF had a lower incidence of dementia (Ruitenberg et al., 2005). In addition, this study also found that in the participants who did not suffer from dementia, faster CBF velocity was associated with lower levels of cognitive decline and a larger hippocampal and larger amygdala volumes (Ruitenberg et al., 2005). The authors suggest that cerebral

hypo-perfusion may be a contributing factor to cognitive decline and the onset of dementia (Ruitenberg et al., 2005).

3.4 Conclusion of Chapter

The current chapter reviewed the cardiovascular and cognitive effects of omega-3 fatty acids both at an epidemiological level and through clinical trial research on humans and animals. It highlighted the need for future research in the area, particularly in a young, healthy adult sample.

Chapter 4 Attention Deficit Hyperactivity Disorder (ADHD)

4.1 Abstract

The current chapter looks at PUFAs in the context of attention deficit hyperactivity disorder (ADHD). This chapter will present a theoretical basis for the possible link between EFAs and ADHD, and provide the rationale for investigation of the effect of essential fatty acids on cognitive performance, cardiovascular performance and brain function using a sample of those suffering from ADHD. It has been hypothesised that essential fatty acid status is related to the prevalence and symptomology of ADHD. This chapter will investigate the connection between essential fatty acid status and ADHD and will outline why EFA supplementation may be a viable treatment option for ADHD, alongside the traditional pharmacological and psychological treatment methods.

4.2 Introduction

As discussed in the previous chapters of this thesis, previous research has suggested that n-3 PUFAs have an effect on cognition (Fontani et al., 2005) and cardiovascular health (Bucher et al., 2002) in the human body, despite the results appearing somewhat ambiguous. In addition to these effects, there is also mounting evidence to suggest that decreased levels of n-3 PUFAs may also be implicated in a wide range of developmental, neurological and psychiatric disorders (Bourre, 2004; Richardson, 2006). One of these disorders of interest is ADHD, with the theoretical basis between ADHD and PUFAs having been explored through research (Gow, Hibbeln, & Parletta, 2015). A growing body of evidence suggests that problems with attention and memory processes in childhood may predict the emergence of psychopathology later in life (McNamara & Carlson, 2006).

What is ADHD?

Attention deficit hyperactivity disorder (ADHD) is a chronic, multifactorial condition, which is characterised by a persistent pattern of inattentiveness, and/or impulsivity and hyperactivity which is not consistent with other individuals at a similar stage of development (American Psychiatric Association, 2000b). It is the most common childhood-onset psychiatric disorder and has high heritability (Sharp, McQuillin, & Gurling, 2009). The symptoms of this disorder typically present between the ages of three and seven and affect sufferers in many different settings including home, work, personal lives and school (American Psychiatric Association, 2000a). These symptoms can have an extensive impact on the quality of life of sufferers. Each sufferer will experience varying levels of attentional problems, hyperactivity and impulsivity, but these symptoms are diagnostically required to affect the day to day functioning of all sufferers to some degree

(American Psychiatric Association, 2000a; Greydanus, Pratt, & Patel, 2007). A broad range of difficulties can manifest from this disorder, with some of the more pervasive difficulties associated with attention, focus and behavioural control. Despite common beliefs, this disorder is not limited to children and adolescents, with approximately half of people who present with childhood ADHD suffering symptoms into adulthood (Biederman, 1998).

History of ADHD

Over the years, several theories have attempted to explain the complex symptomatology of ADHD, ranging from developmental explanations (El-Sayed, Larsson, Persson, Santosh, & Rydelius, 2003), to genetic factors (F. Levy, Hay, McStephen, Wood, & Waldman, 1997) and other explanations as diverse as lead exposure (Nigg, Nikolas, Mark Knottnerus, Cavanagh, & Friderici, 2010) and gut bacteria (Pärtty, Kalliomäki, Wacklin, Salminen, & Isolauri, 2015). Despite providing valuable insight into the disorder, there is no single theoretical perspective that has been able to provide a complete explanation of the disorder. This is further complicated by the complex evolution of the disorder, as it has encountered name changes and major changes in the diagnostic criteria over time (Adler, 2004; Salmelainen, 2002).

It is unknown at exactly which point the symptoms of ADHD were grouped together as a unique disorder, but a large proportion of scientific credit has been given to Sir Alexander Crichton in 1798 and to British physician George Still in 1902 (Barkley & Peters, 2012; Crichton, 2008; Still, 2006). In 1798, Crichton published a book chapter on disorders of attention which has similarities with the DSM-IV-TR criteria for the inattentive subtype of ADHD (Crichton, 2008; Palmer & Finger, 2001) This was built upon in 1902 when Still delivered a series of lectures which were the first to describe a behavioural condition which closely resembles what is known today as ADHD (Barkley & Peters, 2012; Still, 2006). Still noted that in 20 cases of his own observation, children aged between 4 and 13 exhibited symptoms of inattention and overactivity (Barkley & Peters, 2012; Still, 2006). Interestingly, Still also alluded to the gender bias that is present today, stating that he didn't believe the male prevalence of the disorder to be "altogether accidental" (Still, 2006). Overall, it took over a century for evidence of attentional disorders to be recognised as structured syndromes with diagnostic criteria.

The first edition of the DSM published in 1957 did not include ADHD as a disorder in any form (American Psychiatric Association, 1957). It was originally known as hyperkinetic disorder (HKD) in the DSM-II published in 1968 (American Psychiatric Association, 1968), and as attention deficit disorder in the 1980 DSM-III (American Psychiatric

Association, 1980; Spencer et al., 1996). In this edition, two different subtypes of the disorder were defined, ADD with hyperactivity and ADD without hyperactivity. This was changed in 1987 when the revised edition of the manual was released and the disorder was renamed AD/HD, making the disorder more one dimensional (American Psychiatric Association, 1987).

When the DSM-IV was published, there were major changes from the previous edition. This version of the DSM termed the disorder "attention-deficit/hyperactivity disorder" in recognition of the three different subtypes of the disorder. The major difference in this edition of the diagnostic manual was the inclusion of subtypes of the disorder, meaning people could meet the diagnostic criteria if the sufferer experienced either inattention and/or hyperactive and impulsivity symptoms (Spencer et al., 1996). Another major change in the criteria in this edition included the requirement for the symptoms to be present in at least two different settings, a decision that was made to lower the number of false positive diagnoses (Spencer et al., 1996). The DSM IV was revised in 2000, but no changes to the criteria were made. The DSM V was published in 2013, and the main change in this version was the adaption to better include the experience of adults. These changes were based on years of research suggesting that the symptoms of ADHD can persist into adulthood (American Psychiatric Association, 2013a). This is discussed in detail later in the thesis, but it is a step forward in recognising the effect this disorder can have on adults. In previous editions, it was necessary to have six symptoms from either or both of the two criteria. In the latest edition, while children must exhibit six symptoms, adults may receive a diagnosis while only presenting with five. Additionally, while the diagnostic criteria have not changed, additional examples have been included to illustrate behavioural patterns which may be exhibited by adults or older adolescents with the disorder. These changes recognise the differences in the manifestation of the disorder between children and adults, and follow the previous decade of research suggesting the continuity of the disorder into adulthood. Through these nomenclature and theoretical changes, the disorder has developed and grown into what it is today, a complex, multi-faceted disorder that affects a non-trivial subset of the population. The complexities of the disorder make it difficult to develop a clear understanding of the disorder. Therefore, further research into the disorder is warranted, and this thesis will attempt to address this.

There is no universal agreement on the concept of ADHD, as different countries use different diagnostic criteria. These diagnostic manuals are designed to present clinicians with criteria they can use to assess the symptoms of the sufferer and see if they fulfil the

requirements for a diagnosis. At the time of data collection, the two most common resources available to clinicians in order to diagnose ADHD were the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) (American Psychiatric Association, 2000b) and the International Classification of Diseases, 10th revision (ICD-10) (World Health Organization, 2004). The DSM-IV-TR is predominantly used in North America and the ICD-10 is predominantly used in European countries. Differences in the two diagnostic manuals will be discussed below.

Whilst the definitions of ADHD have many similarities between the DSM and the ICD-10, and the two manuals have become closer with more recent editions, there are still some key differences lying in the diagnostic criteria. The most obvious difference is in the naming of the disorder. It is termed ADHD in the DSM-IV-R (American Psychiatric Association, 2000b)and hyper kinetic disorder (HKD) in the ICD-10 (World Health Organization, 2004). There are three other major differences between the two criteria.

Firstly, the presence of both attentional and behavioural symptoms are required by the ICD-10 to confirm the HKD diagnosis. ADHD has different subtypes according to the DSM-IV-TR, with the inattentive subtype and the hyperactive subtype. If both symptom profiles are present at levels that exceed the diagnostic threshold, then the sufferer is diagnosed with the combined type of ADHD (American Psychiatric Association, 2000b).

Secondly, the ICD-10 requires the symptoms of abnormal levels of inattention and overactivity to be present in two different situations (World Health Organization, 2004), whereas the DSM-IV-TR requires "clinically significant impairment in social, academic or occupational functioning" in two or more situations (American Psychiatric Association, 2000b). The difference here is that the ICD-10 requires both inattention and overactivity symptoms to be present, whereas the DSM-IV-TR only requires one of these as long as it is present in two situations and is impairing the life of the sufferer.

The third major difference between the two diagnostic systems is the issue of comorbidity. While the DSM-IV-TR recognises comorbid disorders alongside ADHD (S. I. Lee et al., 2008), the ICD-10 does not allow for multiple diagnoses. If the criteria for another diagnosis is met, the ICD encourages this diagnosis in place of HKD. For example, if someone fulfils the criteria for depression or anxiety, this means they cannot receive a diagnosis of HKD.

In terms of prevalence, research has shown that diagnoses of HKD in the UK were comparatively lower than that of ADHD in the USA (Prendergast et al., 1988). Typical diagnosis rates using the DSM-IV-TR are between 5-10%, whereas the ICD-10 criteria

only yield a diagnosis of around 1-2% (Swanson, Sergeant, et al., 1998). However, what Swanson, Sergeant, et al. (1998) when comparing the prevalence of the disorders, it can be argued that the ICD rate should only be compared with those diagnosed as the combined subtype of ADHD as defined by the DSM. This is because a diagnosis of the combined subtype of ADHD shares similarities to an ICD diagnosis of HKD due to the presence of both behavioural and attentional symptoms. In summary, while there are a lot of similarities between the two diagnoses, the ICD-10 diagnostic criteria are less liberal when compared to that of the DSM-IV-TR. These broader diagnostic criteria lead to a higher rate of diagnosis.

As Australia uses the DSM as the official diagnostic manual for psychiatric disorders, these represent the criteria that will be adopted in this thesis for the disorder. At the time of the data collection for this thesis, the current diagnostic standard used was the DSM-IV-TR. Within the duration of the study, the DSM-V was released. While this thesis will discuss below the changes made between the fourth and fifth editions of the manual, this thesis will focus on the criteria from the fourth edition, as this was the criteria under which the participants were assessed and recruited.

4.3 Diagnostic criteria

As with other mental disorders, the symptoms of ADHD are subjective and based on the perspective of clinicians, parents, teachers or the patients themselves. As yet, no definitive diagnostic criteria have been defined in more objective areas such as cognition, genetics or neuropsychology. In order to avoid the problem of subjective diagnoses, there are strict diagnostic criteria that must be met to fulfil the criteria for ADHD.

DSM diagnosis of ADHD

There are two major categories of symptoms in the current diagnostic criteria contained in the DSM IV-TR, these are inattention symptoms and hyperactivity/impulsivity symptoms (American Psychiatric Association, 2000b), and will be discussed in detail below. There are three types of ADHD as per the DSM IV criteria. They are differentiated by the presence of the two major categories of symptoms, inattention and hyperactivity/impulsivity.

The first type of ADHD is referred to as attention deficit hyperactivity disorder combined type. This occurs when the person fulfils the criteria for both the inattention symptoms and the hyperactivity-impulsivity symptoms. The second category of ADHD diagnosis is the attention deficit hyperactivity disorder predominantly inattentive type. This occurs when the sufferer fulfils the criteria for the symptoms of inattention, but not the criteria

for hyperactivity and impulsivity. The third category of ADHD diagnosis is the attention deficit hyperactivity disorder predominantly hyperactive impulsive type. This occurs when the sufferer fulfils the criteria for the symptoms of hyperactivity or impulsivity, but not the criteria for inattention.

Symptoms of inattention can be varied, and for a diagnosis of the disorder incorporating inattention, a diagnosed person must have six or more of the following nine symptoms, as defined by the DSM IV R (American Psychiatric Association, 2000b) and shown in Table 4-1.

Table 4-1: Diagnostic criteria for ADHD inattentive type (DSM-IV-R).

a	Often fail to pay close attention to details, or make careless mistakes, in schoolwork, work or other activities
b	Often have difficulty sustaining attention in tasks or play activities
c	Often not seem to listen when spoken to directly
d	Often not follow through on instructions and fails to finish schoolwork, chores or duties in the workplace
e	Often have difficulty organising tasks and activities
f	Often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort
g	Often loses things necessary for tasks or activities
h	Often be easily distracted by extraneous stimuli
i	Often be forgetful in daily activities

In the DSM-IV, hyperactivity and impulsivity symptoms are categorised together. For a diagnosis of the disorder incorporating impulsivity and hyperactivity, a person must have six or more of the following symptoms, as defined by the DSM-IV-R (American Psychiatric Association, 2000b) and shown in Table 4-2 below.

Table 4-2: Diagnostic criteria for ADHD hyperactive/impulsive type (DSM-IV-R).

	Hyperactivity symptoms
a	Often fidget with hands or feet or squirm in their seat
b	Often leave seat in classroom or in other situations in which remaining seated is expected
c	Often run about or climb excessively in situations where it is inappropriate
d	Often have difficulty playing or engaging in leisure activities quietly
e	Often be "on the go" or act as though they are driven by a motor
f	Often talk excessively
	Impulsivity symptoms
g	Often blurt out answers before questions have been completed
h	Often have difficulty waiting in turn
i	Often interrupt or intrude on others

The DSM-IV-R presents three central criteria that must be present in order to make a diagnosis of ADHD (American Psychiatric Association, 2000b). Firstly, the symptoms must be presently occurring, and have been present for at least six months. These symptoms must also be maladaptive, and have an effect on the daily life of the person (American Psychiatric Association, 2000b).

The second critical criteria that must be met for a diagnosis of ADHD is that some of the hyperactive or impulsive symptoms must have been present prior to the age of 7 (American Psychiatric Association, 2000b). An official childhood diagnosis is not required, but there must be some evidence of symptoms before age 7. This evidence can be obtained through school reports, asking parents or teachers, or other retrospective methods. However, the subjective nature of this data, along with the delay between the symptoms occurring and the recall of them, may be a reason to question the validity of

these methods. Many individuals are diagnosed after the symptoms have been present for a number of years, clouding the exact time the symptoms first presented.

The third central criteria that must be met for a diagnosis of ADHD is that the impairment from the symptoms must be present in more than one setting, with the presentation of symptoms in one setting alone not enough to provide a diagnosis. These settings could include school, work, social or home life. If the symptoms are affecting the person in more than one of these settings, then a diagnosis of ADHD may be valid.

While it may seem that everyone experiences a degree of these symptoms at one time or another, if they are a symptom of ADHD they are consistent and affect the persons everyday life. The symptoms of this disorder are on a spectrum, meaning that individuals can present with some symptoms of the disorder at any point in time and in varying degree, but it is only when they have a consistent effect on daily existence that a diagnosis can be made. Symptoms can exist from non-pathological through pathological proportions in the population but need to be pervasive over time to warrant a diagnosis of the disorder.

Differences Between DSM-IV-TR and the DSM-V

The diagnostic criteria for the DSM-IV-TR and the DSM-V are similar (American Psychiatric Association, 2013c). There are however some important changes that will be discussed below, mainly addressing the issue of the symptoms across the lifespan. The 18 symptoms are the same in both manuals, and are still divided into the two symptom categories of inattention and hyperactivity/impulsivity. Examples have been added to the criterion to make them applicable across the lifespan, and not just in childhood and adolescence. Individuals less than 17 must still suffer from 6 of 9 inattentive and/or hyperactive impulsive symptoms in order to receive a diagnosis, but only 5 or more symptoms are required for individuals aged 17 or above. This reflects the DSM-V stating that a lower symptom threshold is needed for adults than children in order to make a reliable diagnosis.

There have also been changes in terms of multiple settings required for the symptoms to occur. In the previous edition, impairment must be present in two or more settings. Therefore, as well as needing to be present in more than one setting, they also had to affect the child's functioning in both of these settings. The DSM-V has changed this to "several inattentive or hyperactive-impulsive symptoms are present in two or more settings" (American Psychiatric Association, 2013b). The difference is that in the new

criteria, the symptoms must be present, but the criteria do not require them to impair the functioning of the individual in all of these contexts.

Additionally, the criterion for age of onset has been changed from "symptoms that caused impairment were present before age 7 years" to "several inattentive or hyperactive-impulsive symptoms were present prior to age 12" (American Psychiatric Association, 2000a, 2013b, 2013c). This is a more lenient criterion, recognising that onset age can differ between individuals.

These changes in criteria are a positive change in the right direction for widening the criteria to include sufferers of all ages. This has been achieved by the inclusion of examples of symptoms in adults, along with a slight relaxation of the criteria in order to receive a diagnosis in older age. However further research needs to be conducted in order to determine how the criteria could better be suited to incorporate the entire lifespan.

4.4 Co-morbidities of ADHD

A comorbidity can be defined as a medical condition that co-occurs with another and can be seen as the simultaneous occurrence of two separate disorders, which are independent of one another. These co-morbid disorders can have an effect on the symptoms, diagnosis and treatment of ADHD (Biederman, Newcorn, & Sprich, 1991) so it is important that comorbidities are considered when looking at ADHD. ADHD regularly presents co-morbidly with other psychiatric disorders, and this frequent co-morbidity has become part of its profile. Many psychiatric disorders frequently present alongside ADHD, and the disorder has been linked with higher incidences of anxiety, depression, and higher schizophrenia symptoms among others (Biederman et al., 1991; Richardson, 2006). Research has found that of children who meet the full diagnostic criteria for ADHD, 87% of them have a comorbid disorder and 67% of them had at least two comorbid disorders (Kadesjö & Gillberg, 2001), showing the prevalence of comorbidities within the disorder. While there is a high rate of comorbidity, some research has estimated approximately 40% of sufferers of adult ADHD will not have an co-morbid condition (Spencer et al., 1996).

The high prevalence of comorbid disorders with ADHD causes many difficulties, as a diagnosis of ADHD cannot be made if the symptoms are better accounted for by the presence of another disorder (American Psychiatric Association, 2000b). As a result of these co-morbidities, it has been suggested that ADHD may simply be a precursor for other conditions rather than a separate diagnosis (Elliott, 2002). This has been refuted

though, with researchers stating that if this were the case, then there would be few if any cases of ADHD without an additional diagnosis.

If there are comorbid conditions that present alongside the ADHD, this may exacerbate the symptoms of the ADHD or vice versa. Additionally, the existence of one comorbid disorder has been found to heighten the risk of developing additional comorbid disorders(D. F. Connor et al., 2003; Waxmonsky, 2003). Symptoms of these conditions may affect treatment compliance and response, and therefore interfere with the efficacy of the treatment. When diagnosing, and treating ADHD, it is important to identify and manage any comorbidities that may influence the primary diagnosis.

When evaluating ADHD and the presence of co-morbid disorders, it is important to determine whether one primary diagnosis can account for the more severe and debilitating symptoms (Faraone, 2007). If a primary condition can better explain the symptoms someone is suffering, then this condition should be diagnosed. However, one should be careful of overarching diagnoses. If a child meets the criteria for more than on condition, they are labelled as the more severe diagnosis. This does not mean the symptoms of the less severe diagnosis disappear, simply that the primary diagnosis becomes the treatment focus. If both conditions are contributing to the impairments, both diagnoses should be valid and treated (Faraone et al., 2007). These co-morbidities can have even more of an effect on the adult form of the disorder, due to adults having added responsibilities and less guidance and supervision.

4.5 Prevalence of ADHD

With estimates that ADHD is present in 1-10% of school aged children (American Psychiatric Association, 2000b),it is a major public health issue in the world as it has a detrimental effect on society in many ways, including direct economic and associated health costs (Spencer et al., 1996). There is no clear consensus in regards to symptom severity and this is further complicated through the use of different diagnostic systems being used in different parts of the world as discussed in section 4.3.

Visser, Lesesne, and Perou (2007) found that 7.8% of American children aged between 4 and 17 have a reported diagnosis of ADHD , however there is a big variation in estimates with some estimates being as low as 3.9% (Burd, Klug, Coumbe, & Kerbeshian, 2003). When estimating the prevalence of ADHD, one must consider the limitations of the method of estimation and how this explains a lot of the variance. The criteria used for diagnosis must be considered. As would be expected, the stricter the criteria used, the lower the number of diagnoses, but that some of the variability in the number of

diagnosed cases comes from the variability in the symptoms that come from the disorder (Richardson, 2006). As symptoms of ADHD are often extremes of normal behaviour, it is difficult to determine the exact threshold between normal and abnormal behaviour (Richardson, 2006). While diagnostic criteria do exist, it can be difficult to establish the exact point where the behaviour becomes dysfunctional to the wellbeing of the person. This leads to a large variation in population estimates, as is demonstrated through research.

Table 4-3 and Table 4-4 have been adapted from Swanson, Sergeant, et al. (1998), and provides an overview of the prevalence of ADHD rates across different countries using different diagnostic methods.

Table 4-3: ADHD diagnostic prevalence rates across different countries (Adapted from Swanson et al., 1998).

Subtype and DSM diagnosis	Country	Age (years)	Gender	Prevalence
DSM-III ADD/H	USA	6-9	M	8%
DSM-III ADD	New Zealand	11	M/F	7%
DSM-III ADD/H	Canada	4-16	M/F	6%
DSM-III ADD/H	Puerto Rico	4-16	M/F	9%
DSM-III ADD/H	United Kingdom	6-8	M	5%
DSM-III-R AD/HD	Hong Kong	7	M	9%

Table 4-4: HKD diagnostic prevalence rates across difference countries (adapted from Swanson et al., 1998).

Subtype and DSM diagnosis	Country	Age (years)	Gender	Prevalence
ICD-9 HKD	Sweden	5-12	M	2%
ICD-9 HKD	Germany	8	M/F	4%
ICD-9 HKD	Germany	13	M/F	2%
ICD-9 HKD	UK	7	M	2%
ICD-9 HKD	Hong Kong	7	M	1%

Most of the research into ADHD originates from Western nations, predominantly the United States, so the reported prevalence rates are heavily reflective of this area. A Korean study on a sample aged between 5 and 16 found that 76.2% of the sample population had a co-morbid disorder, with oppositional defiant disorder being the most common comorbidity (Byun et al., 2006; Gillberg, 1998). To the author's knowledge, no research has been conducted investigating the prevalence of the disorder in an exclusively Australian population. However, due to same criteria being used across the countries, and the similarity in culture and society between the United States and Australia, it can be reasonably assumed that the prevalence will be similar. It must also be noted that there is a lack of reliable ADHD research in developing countries, and consequently no accurate estimate of the prevalence of the disorder in these countries. With no data to examine for this population, it has been suggested that the prevalence and impact of the disorder in developing countries may be similar to that in the Western world (Remschmidt et al., 2005). Polanczyk, De Lima, Horta, Biederman, and Rohde (2007)conducted a systematic review comprising of 102 studies with 171,756 subjects from all world regions. North America was found to differ significantly in ADHD symptoms from Africa and the Middle East, but did not differ from Europe. The authors suggest that this indicates geographic location only plays a limited role in the variance of estimates of the presence of ADHD globally (Polanczyk et al., 2007). Further research is needed in this area to determine if this is the case. More cross-cultural research will aid accurate diagnosis and treatment wherever the disorder occurs, and reduce the global impact of the disorder.

While there are clear criteria that need to be met before a diagnosis can be made, there are still a few areas of contention. The criteria are quite rigid and do not consider the differences in developmental paths. As most diagnoses are made at a critical time in a child's development, there is naturally a large amount of variation in behaviour that in turn affects the presentation of the symptoms of the disorder. While these factors may seem individually small, they can cause errors in diagnosis and their combined effect can affect the reliability of the diagnoses made. This issue is exacerbated, as evidenced in Table 4-3 and Table 4-4, by the large amount of variance in the data collected in the research that has been conducted. All the studies varied in terms of age and gender of participants and this may have contributed to the variation in prevalence. This highlights the need for diagnostic criteria to take into account developmental stages and the gender of the participants it is being used to diagnose.

4.6 Gender Differences in ADHD

A recognition of the gender difference in ADHD is essential to understanding the disorder. It has been estimated that the disorder is at minimum three times more prevalent in males than females, with a 10:1 ratio being detected in clinical populations and a 3:1 ratio present in community based samples (Biederman et al., 2002; Gershon, 2002). Research has suggested that this may be in part due to the diagnostic criteria used, with some suggesting the gender ratio will never reach equality without a change in the criteria (Arnold, 1996).

Despite these figures, this may not necessarily be indicative of a gender bias within the ADHD population. It must be noted that referrals to clinicians are typically for external symptoms such as aggressiveness or problem behaviour and these are more typical in males than in females (Gaub & Carlson, 1997). Females with ADHD may exhibit symptoms that are more likely to be overlooked due to them being typically less noticeable, and as such the gender bias may be exaggerated. This may lead to the females who are referred to clinicians exhibiting particularly disruptive behaviour that is not typical of the majority of females with the disorder.

Additionally, many claims about ADHD being more prevalent in males come from paediatric populations, such as the research from Biederman et al. (2002) and (Gershon, 2002). Gershon (2002) acknowledge that based on community samples, a larger proportion of females could meet the criteria despite rarely being included in clinical studies. This lack of data is problematic, as the underdiagnoses of the disorder could lead to long term health problems such as social, academic and emotional difficulties being left untreated. The problems with obtaining a representative sample are echoed by other research, with V. Simon, Czobor, Bálint, Mészáros, and Bitter (2018) finding in a meta-analysis that convenience sampling is a problem in the area. This study found that although the sample size of the meta-analysis was large (5307 participants), the majority of studies recruited by convenience, and the samples were not representative. The current study did not adopt a convenience sampling approach, rather the researchers aimed for a 3-1 ratio of males to females, based on research by Biederman et al. (2002). As the current study used a combination of clinical and community sampling, the ratio was closer to 1-1.

4.7 Societal Impact of ADHD

Evidence has suggested that the cost of ADHD to society is a large one (Burd et al., 2003). One study used population based data from the U.S state of North Dakota to estimate the

prevalence and cost of treatment of children with ADHD to the U.S economy (Burd et al., 2003). From a population of 7745 children, a mean prevalence of 3.9% was detected. The annual cost of care for children with ADHD in North Dakota was placed at $5.1 million, which is 5.6% of the total annual health care cost for children (Burd et al., 2003). If this was extrapolated to the entire United States, it would be estimated that the cost of care attributable to ADHD would be $2.15 billion annually (Burd et al., 2003). Further research may be able to reduce this amount, lessening the effect the disorder has on general society.

4.8 Aetiology of ADHD

The aetiology of ADHD is thought to be multifactorial (Clarke, Heussler, & Kohn, 2005; Franke, Neale, & Faraone, 2009). There are currently no objective biological markers associated with ADHD, and at the present time it appears as though both genetic and environmental factors play a role in the aetiology of the disorder (Richardson, 2006). The disorder is further complicated by the heterogeneity of symptoms between sufferers and the high rate of co-morbidity it shares with other disorders.

Previous research has suggested that deficiencies in EFA levels may be related to hyperactivity in children (Colquhoun & Bunday, 1981), a key symptom of ADHD. Whilst the exact mechanism of this relationship is not yet fully understood, many theories have been suggested, including malabsorption of EFAs or problems with metabolising linoleic acid, all of which would lead to higher EFA requirements within this population of hyperactive children (Colquhoun & Bunday, 1981). Following this, it has also been suggested that dietary supplementation of EFAs may alleviate this hyperactive behaviour (Stevens et al., 1995).

It has been suggested that many genes contribute to the disorder in various ways in the presence of certain environmental conditions (Franke et al., 2009). While it is recognised that behaviour is not directly controlled by genes (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008) there does appears to be a genetic component of ADHD, with it being highly hereditary (Salmelainen, 2002). Research has found that first degree relatives of children with ADHD have a significantly higher prevalence of ADHD than matched controls, and this has been found in both genders (Faraone, Biederman, Spencer, et al., 2000). Twin and family studies have shown that there appears to be a large genetic overlap associated with the disorder, but little family environmental effect (Sharp et al., 2009). While research has indicated there may be some heritability factors associated with the aetiology of ADHD (Faraone, Biederman, Mick, et al., 2000; Sharp et al., 2009), it does not provide a full explanation of the genetic aspect of the disorder.

A possible genetic explanation for ADHD has been the suggestion that it may occur due to abnormal levels of neurotransmitters, with specific genes in the dopaminergic system thought to be involved (Tannock, 1998). One of the primary factors that underlies ADHD is poor behavioural inhibition (Barkley, 1997). This lack of inhibition can then lead to impeded performance in other secondary neuropsychological domains, such as working memory, speech internalisation, mood regulation and motor control (Barkley, 1997). If these areas of the brain are delayed in development, as often is the case in ADHD, low levels of neurotransmitters such as dopamine and noradrenalin may result. Genes from the dopaminergic system have been found to be involved in the aetiology of ADHD (Cook Jr et al., 1995; Tannock, 1998).

Despite the vast amount of research that has been undertaken on ADHD, there has been some debate about the diagnostic validity of ADHD, partially due to the many changes in criteria over time (Rhodes, Coghill, & Matthews, 2006). As with most other mental disorders, the symptoms of ADHD can be viewed on a spectrum and it can sometimes be difficult to define the difference between clinical diagnosis and a variation in the normal population. The difference between the two lies in the level of impairment caused by the symptoms.

The perceived ambiguity of the disorder is heightened by the subjective interpretation of the diagnostic criteria. This ambiguity may also be a result of other factors, including co-morbid disorders, misunderstandings of the diagnostic criteria, or the applying of criteria where the children do not reach the threshold. This is further complicated by the change in diagnostic criteria as the different manuals update editions.

An international consensus statement on ADHD (Barkley, 2002) stated that the idea that ADHD does not exist is "simply wrong" due to the scientific evidence indicating it is a genuine disorder - a statement signed by many prominent scientists in the field. However, until there are definitive objective makers for ADHD which are neural, cognitive or genetic, there is some reason for a degree of scepticism. Objective research, such as systematic reviews of diagnostic criteria is made harder by this lack of definitive markers of the disorder. It can be seen that more targeted, properly designed research is needed in this area to gain a better understanding of the concept of the disorder.

Adult ADHD

While there is some debate about the validity of ADHD as a disorder in children, this debate is magnified when it come to the disorder in adults. While the disorder for the most part remains a disorder of childhood, there is growing recognition of its continuance

throughout the lifespan, as evidenced by changes in the DSM-V. It must be noted that the vast majority of research in this area has been conducted on children and there is comparatively very little research to investigate the disorder in adults. As ADHD symptoms continue to affect sufferers throughout their lifespan, it is the aim of the present study to investigate the disorder in young adults.

Clinical follow up studies have shown that people who suffer from ADHD in childhood often continue to suffer from symptoms into adulthood (Gittelman, Mannuzza, Shenker, & Bonagura, 1985), with studies showing approximately half of children diagnosed with the disorder will continue to suffer into adulthood (Biederman, 1998). Studies examining the continuity of symptoms suggest that the hyperactive symptoms may decline over the lifespan, but the inattention aspect of the disorder is more likely to continue into adulthood (Mannuzza, Klein, & Moulton, 2003). One study that evaluated ADHD symptoms in a large group of adults looked at psychiatric co-morbidity, age and gender in reference to symptoms of ADHD (Totaro, Marini, Cannarsa, & Prencipe, 1992). In a sample of 149 clinical patients with ADHD, they found that inattentive symptoms were present in over 90% of the sample (Totaro et al., 1992). When assessed as adults, 37% of participants were categorised as the inattentive subtype of ADHD, whereas only 3% of the population were found to suffer from the hyperactive, impulsive subtype. In addition, 56% of the sample were found to suffer from the combined subtype, and 5% were not otherwise specified (Totaro et al., 1992). This data suggests that while ADHD symptoms can be seen to decrease over time, the symptoms of inattention appear to be more pervasive than the hyperactive symptoms(Mannuzza et al., 2003).

Despite the research suggesting that ADHD does persist into adulthood (Kooij et al., 2005), there is limited research investigating the prevalence of the disorder in late adolescence and adulthood (American Psychiatric Association, 2000b). Within the research that has been conducted, large variations in estimations predicting the disorders persistence into adulthood have been detected. Estimates of prevalence vary, ranging from 2% (Spencer et al., 1996) to 3.4% (Fayyad et al., 2007). Some research suggests as many as 60% of individuals with childhood onset ADHD continue to suffer from symptoms into adulthood, regardless of whether these symptoms manifest into a full diagnosis of the disorder (Elliott, 2002; Panza et al., 2004). The wide variety of estimates is due to multiple factors, including the limited amount of data to base this estimate on, and the heterogeneity of the disorder. However, even if the lower estimates are accurate, and only 2% of adults suffer from this disorder, it is still a relatively common disorder

and more epidemiological and clinical research is needed to investigate the outcomes of this disorder.

Controversies of adult ADHD

Despite ADHD being a recognised, widely researched disorder in children, there is little research on the disorders clinical presentation in adulthood, and the diagnosis remains controversial (Elliott, 2002). One reason for this is the difficulty of getting accurate information to validate a retrospective diagnosis. In order for a retrospective diagnosis to be made, a clinician needs to establish that the symptoms occurred before age seven. Normally, an adult who is presenting with the symptoms of ADHD will have a history of behavioural problems in childhood, such as discipline issues and educational difficulties (Willie et al., 2011). This is normally through a combination of methods, including a clinical interview and observer reports filled in by parents. The problem with these methods however, is the amount of time between the symptoms occurring and the recall of them, which can often be 20 to 30 years.

It has been suggested that the symptoms of ADHD do not disappear in adulthood, but are managed differently. As an adult, the behaviours associated with the disorder become more noticeable and may have a larger impact on the functioning in an adult setting. For example, while yelling out in class is not acceptable at school, it is even more inappropriate in a setting such as the workplace, and it may have more severe consequences than what would be present in a school setting. For this reason, adults with ADHD have often learned to adjust their lifestyles to better manage their symptoms. Adults with ADHD often try to offset the impairment caused by the disorder by adopting compensatory behaviours, for example becoming overly dependent on items such as diaries and personal digital assistants (Adler, 2004).

4.9 Neuroanatomical Features of ADHD

Neurophysiology of ADHD

One approach to understanding mental disorders is to take a psychophysiological approach, which focuses on underlying brain processes and how they affect behaviour. The majority of research on ADHD has focussed on executive function, inhibitory control and inattention. This is due to inhibitory control and inattention being key components of the diagnostic criteria (see Table 4-1 and Table 4-2). Deficits in inhibition have been associated with damage to the frontal lobe (Dimitrov et al., 2003). As inhibitory control is one of the core deficits of the disorder, it was hypothesised that people who suffer from ADHD may have anatomical differences in the frontal lobe.

fMRI Research in ADHD

As with most areas in ADHD, the fMRI literature is generally limited to child and adolescents, meaning that the developmental stage of the brain must be considered when results from imaging studies are examined. Most of the fMRI research in the area has focussed on anatomical correlates of attention networks and the impairment of executive functions such as inhibition, working memory and motor execution (M. Schneider, Retz, Coogan, Thome, & Rösler, 2006). The anterior cingulate cortex plays a central role in attentional processing.

Neuroanatomical Features

Studies of the anatomy of the brains of people with ADHD have found overall reductions in brain size when compared with age and gender matched controls (Castellanos et al., 1996). In a sample of 57 boys with ADHD and 55 age and gender matched controls, the ADHD subjects had 4.7% less total cerebral volume than the controls (Castellanos et al., 1996). This finding was also supported in a smaller study of 12 ADHD boy participants and 12 age matched controls (Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002), where the ADHD participants were found to have an 8.3% smaller cerebral volume. The authors suggest that the deficits in inhibition which present in ADHD may be a result of closely located but still separate abnormalities in parallel circuits (Mostofsky et al., 2002). Interestingly, the volume reduction has been found to more marked in the right hemisphere (M. Schneider et al., 2006).

Neuropsychological findings suggest the behavioural signs of ADHD may result from underlying deficits in response inhibition, delay aversion and executive functioning (Krain & Castellanos, 2006). Multiple studies have indicated that children and adolescents with ADHD have deficits in either or both selective and sustained attention tasks when compared to a matched control sample (Berger & Cassuto, 2014; Yang, Liu, Xu, & Liu, 2012)

Additionally, some reviews have implicated abnormalities in the frontostriatal networks as being involved in the mechanisms of ADHD (Swanson, Castellanos, Murias, LaHoste, & Kennedy, 1998; Tannock, 1998). As the anterior cingulate cortex has been identified as an area of importance to the frontostriatal cortex (Bush et al., 1999) and plays a central role in attentional processing, it was hypothesised that the ACC may play a role in ADHD. Dysfunctions in this area have been identified as contributing to core features of ADHD such as inattention and impulsivity (Bush et al., 1999). Bush et al. (1999) conducted an fMRI study investigating whether adults with ADHD would show performance deficits in a counting Stroop task when compared to matched control participants. In a small study

conducted on 16 unmedicated adult participants, 8 with ADHD and 8 controls, Bush et al. (1999) found ADHD participants showed significantly lower ACC activation than matched control participants when performing a counting Stroop during fMRI . While the Stroop interference effect was observed in both groups, the activation of the ACC area was significantly higher in the control group when compared to the ADHD participants. This data supports the theory that the ACC area in dysfunctional in people with ADHD, and the different activation patterns detected in the ADHD participants suggest that the dysfunction was regionally specific, and not to a global inability to complete a cognitive task (Bush et al., 1999). The authors note that this was not due to a generally poor neuronal responsiveness, as the ADHD subjects did activate a frontostriatal network (Bush et al., 1999).

There is also evidence in the literature to suggest that a negative correlation exists between impulsivity/hyperactivity and ventrostriatal activity in the brain (Scheres, Milham, Knutson, & Castellanos, 2007; Ströhle et al., 2008). When completing a monetary incentive delay task, participants with ADHD displayed decreased activation in the ventral striatum when they were anticipating the financial gain, and increased activation of the orbitofrontal cortex in response to the outcomes of the task (Ströhle et al., 2008). Interestingly, the ventral striatum activation was negatively correlated with self-reported scores of symptoms of hyperactivity and impulsivity (Ströhle et al., 2008).

A meta-analysis conducted on 11 studies found the volume of the grey matter of the basal ganglia is reduced in children with ADHD, and these differences appear to diminish over time (Frodl & Skokauskas, 2012). Additionally, the study found that adults with ADHD were characterised by volume reduction in the ACC (Frodl & Skokauskas, 2012). Further analyses suggested that studies that included more subjects that had been previously treated reported less change in the ACC (Frodl & Skokauskas, 2012). The researchers suggest that treatment may have a positive effect on changes in the brain, but further research is required to investigate this hypothesis. Additionally, the authors acknowledge that all of the studies in the analysis were cross-sectional and it is not possible to draw definitive conclusions on the longitudinal effects between childhood, adolescence and adulthood based on this design.

A study conducted on 36 male participants, 18 with an ADHD diagnosis and 18 age-matched control participants investigated structural brain differences between ADHD and control participants (Bonath, Tegelbeckers, Wilke, Flechtner, & Krauel, 2016). Significant differences were found in regional grey matter measures in the cerebellum, occipital region, ACC, hippocampus and the amygdala. In all of these areas, the control

participants had increased grey matter measures relative to the ADHD group. Additionally, correlational analysis found a significant positive correlation between grey matter volume of the ACC and results on tests of selective attention. While this relationship was present across all participants, separate analyses revealed that this positive correlation was driven by the ADHD cohort, and was not present in the control group alone. This result suggests that poorer performance in selective attention tasks is related to the lower ACC grey matter volume found in the ADHD participants. The authors suggest that these findings indicate structural differences in the brain may underlie the attentional issues associated with the disorder (Bonath et al., 2016).

As with most research in ADHD, the vast majority of research is conducted on children (M. F. Schneider et al., 2010). As such, it remains unknown whether network activation differences in ADHD participants in comparison to control differ between children and adults with the disorder (M. F. Schneider et al., 2010). If this is the case, then it is possible that the remission of ADHD symptoms may run parallel with a change in brain function closer to that of control participants (M. F. Schneider et al., 2010). This view is supported by fMRI research which found that hypofunction in the dorsal anterior midcingulate cortex was reversed following methylphenidate administration (Bush et al., 2008). This possibility should be considered with caution however, as it is possible that adults with ADHD may be more capable of compensatory brain mechanisms that children and adolescent brains may not be able to achieve (M. F. Schneider et al., 2010).

Imaging research in ADHD participants has also investigated whether there were structural differences between the brains of people with ADHD and matched controls. In a sample of 3242 participants, a cross-sectional meta-analysis analysed T1-weighted MRI data across the two groups. This data suggests that patients with ADHD do have structural brain differences in the amygdala and hippocampus when compared to matched control brains. In addition, the data did not show significant differences between medication-naïve and medicated groups (Hoogman et al., 2017). The authors hypothesise that the brain maturation delay theory of ADHD can be extended to include structural differences. While these results may seem interesting, care needs to be taken in the interpretation of the results due to the cross-sectional nature of the research.

4.10 Treatment of ADHD

Researchers are yet to ascertain a precise understanding of the mechanisms of ADHD, and at the present time there are many different treatment strategies that are being used (Salmelainen, 2002). Most research concludes that management of the condition should adopt a multifaceted approach in order to reflect the complexity of the condition

(Greydanus, Patel, & Pratt, 2006). Before any treatment plan is adopted, it is important to ensure that any co-morbidity is considered.

The two main treatment strategies adopted at the present time are the psychological approach and the pharmacological approach; however there is growing interest in alternative approaches including the use of EFA supplementation. The different methods are often combined to globally target the disorder. This thesis will examine each of the treatment methods, and provide a discussion of each methods strengths and weaknesses and why they are currently in use.

Psychosocial Treatment

There are a wide variety of psychosocial interventions that may be used in the treatment of ADHD, and these are often used in conjunction with pharmacological interventions. Psychosocial treatments address social emotional and behavioural functioning and include, but are not limited to, psychotherapy, cognitive behaviour therapy, support groups and parent teacher training (Greydanus et al., 2007). The evidence for these types of interventions is inconsistent, with a range of data collected, ranging from dubious scientific method to controlled clinical trials. It is important that all sufferers of the disorder are presented with all the treatment options available so they can make an informed decision. While psychosocial treatment methods combined with medication have been shown to produce slightly better positive functioning outcomes than medication alone (The MTA Cooperative Group, 1999), they have not been found to be as effective as stimulant medication when they are compared as single treatment approaches (Van der Oord, Prins, Oosterlaan, & Emmelkamp, 2008).

There is also the issue of compliance with treatment plans and this is especially apparent when it comes to adults with the disorder. In order for them to succeed, there is a large assumption of motivation of the participant (Salmelainen, 2002). Due to this barrier, many of the non-pharmacological treatment plans, such as behavioural parent training and behavioural classroom interventions, are not suitable for all people who suffer from the disorder. These psychosocial treatments are based on training which involves a repetition of behaviours, and they work best in a setting with regular routines such as a school setting. While these treatment strategies may be useful for children who live in a more structured environment, it may not be of as much assistance to adults suffering from the disorder. This is due to the symptoms of the disorder, such as inattentiveness and difficulty with organisation, possibly causing difficulties in implementing the necessary steps for this treatment to work. The higher the intensity of these interventions, the more effective the interventions appear to be (Salmelainen, 2002) however more intensity

requires a higher need of commitment and this can be difficult in an adult ADHD sample. If the person suffering ADHD is unable or not willing to implement these strategies, then it is less likely the strategies will be successful.

Pharmacological Treatment

As a result of these findings, for decades the most common form of treatment for ADHD has been a pharmacological approach (Lerner & Wigal, 2008). There is over 60 years of evidence suggesting the beneficial effects of medication on ADHD symptoms, primarily stimulant medication which has been shown to have a positive effect on hyperactivity, impulsivity and attention (Greydanus et al., 2006).

Stimulant drugs primarily exert their effect on the central nervous system, acting through the dopaminergic and noradrenergic pathways (Greydanus et al., 2006). The most common stimulant medications used to treat the disorder are methylphenidate and dexamphetamine (Poulton, 2006). While these medications are effective in managing the symptoms of the disorder, it is important that researchers understand the implications of these drugs, especially as they are increasingly prescribed to the developing brains of children (Salmelainen, 2002). The long-term effects of these drugs are relatively unknown, and longitudinal research is necessary to further understand the implications of regular intake of these drugs over an extended period of time. In 2006, 5 million Americans were prescribed psychostimulant medication, with 3.5 million of these prescribed for people between the ages of 3 and 19, and 1.5 million between the ages of 20 and 64 (Greydanus et al., 2006). In 2000, five times as many children and adolescents started taking stimulant medication as in 1990 (Salmelainen, 2002). Researchers have found that 7.8% of American children aged between 4 and 17 have a reported diagnosis of ADHD and of these 56.3% of them were taking medication at the time of the study (Visser et al., 2007).

Psychosocial vs Pharmacological Treatments

There are many reported side effects reported to coincide with the beginning of stimulant treatment, including reduced appetite, weight loss, and delayed sleep onset among others (Lerner & Wigal, 2008; Wolraich, McGuinn, & Doffing, 2007). Other adverse effects may be of a psychological nature, such as anxiety and increased irritability (Lerner & Wigal, 2008).

Recently, there has been concern expressed over the potential cardiovascular effects on children taking stimulant medication for ADHD, as stimulant usage has been linked to cardiovascular effects such as increased blood pressure and heart rate, and this trend has

been found in follow up studies (Wilens, Biederman, & Lerner, 2004) and randomised placebo controlled trials (Samuels, Franco, Wan, & Sorof, 2006). However some contradictory evidence has also been published, with some short term studies finding that the changes in pulse rate and blood pressure caused by stimulant usage was clinically insignificant (Findling, Short, & Manos, 2001). Additionally, other studies have also found while there were no statistically significant changes detected in diastolic blood pressure or heart rate over a duration of 2 years, a small but statistically significant change in systolic blood pressure was detected(Wilens et al., 2004). While the exact mechanisms of this relationship are not yet fully understood, some research suggested that cardiovascular risk is increased through the stimulants acting as sympathomimetic agents (Nissen, 2006). As there is a large amount of contradictory evidence published, more research is needed in the area.

For these reasons, the issue of pharmacological treatments versus psychosocial interventions remains an issue in the treatment of ADHD. Research that has been conducted in this area is varied, with some advocating the use of stimulant medication, others suggesting the long term risks associated with this treatment method are too high and others criticising the methodologies undertaken and the lack of reporting of adverse findings (Jadad, Boyle, Cunningham, Kim, & Schachar, 1999). Considerable care needs to be taken when prescribing psychostimulants as a treatment method for ADHD. Psychostimulants are classified as schedule II drugs, as along with medicinal benefit, they also have the potential to be abused (Greydanus et al., 2006).

Alternative Treatments

Due to the problems associated with prescription drug treatment of ADHD, research is searching for alternative treatments for the disorder. Dietary interventions have been used in the treatment of ADHD (National Collaborating Centre for Mental Health, 2009) and there is growing evidence to suggest that fatty acid supplementation could be a valuable treatment method for ADHD. As with most areas of the disorder, the research conducted has focussed on children and adolescents, but there is a small amount of literature on the use of EFAs as a treatment in an adult population. A study of young boys found that subjects with ADHD had significantly lower concentrations of key essential fatty acids than the control subjects and this was found in both red blood cell total lipids and plasma polar lipids (Stevens et al., 1995). Interestingly, these results occurred despite the finding that the ADHD group consumed significantly more dietary PUFAs as compared with healthy controls. The authors of this study did not make any inferences from these findings, apart from suggesting that it is unlikely to be due to a primary deficiency of these

acids, as the ADHD participants had a higher dietary intake of EFAs. This cannot be entirely relied upon however, as there was incomplete nutritional information available on some foods, especially n-3 levels.

One of the advantages of EFA supplementation as a treatment for ADHD is that they can be regarded either as a sole type of therapy, or as a complementary therapy alongside pharmacological treatments. Due to the lack of side effects of EFA supplementation, there are few identifiable risks associated with this method, so it seems logical to investigate EFA supplementation as a possible treatment method. The basis for the idea that EFAs might work as a treatment strategy for ADHD comes largely from animal research (Chalon, 2009).

The connection between neurodevelopmental disorders, such as ADHD, with n-3 deficiency, was first proposed in the 1980's where researchers hypothesised that a lack of essential fatty acids in the diet of children were a causal factor in ADHD (Colquhoun & Bunday, 1981). This came from the observation that children who exhibited hyperactive symptoms reported being thirstier than children who didn't exhibit the symptoms (Colquhoun & Bunday, 1981). As thirst is a primary symptom of EFA deficiency, it was hypothesised that there was a connection between the hyperactive symptoms and EFA (Colquhoun & Bunday, 1981). However, thirst is not specific to EFA deficiency and could have occurred for numerous other reasons.

Other research also supported the hypothesis of a link between ADHD and EFA levels. A study which measured plasma fatty acid levels in children with hyperactivity symptoms and a matched control sample found that various PUFA levels, including DHA, were lower in the children with hyperactivity (Mitchell, Aman, Turbott, & Manku, 1987). This has since been supported by numerous studies (Burgess, Stevens, Zhang, & Peck, 2000; Stevens et al., 1996). As there is no evidence suggesting that dietary intake of n-3 PUFAs is lower in children with ADHD as compared to matched controls (Ng, Meyer, Reece, & Sinn, 2009; Stevens et al., 1995), this suggests the differences are due to altered metabolism of these acids. Further research found that physical symptoms of EFA deficiency presented more commonly in children with ADHD than in matched controls and also that high scores on a rating scale of ADHD symptoms were associated with low n-3 plasma levels (Stevens et al., 1995).

In 2000, Richardson and colleagues noted clinical similarities between sufferers of ADHD and people with n-3 PUFA deficiencies (Richardson & Puri, 2000). They suggested that some features of ADHD reflect underlying essential fatty acid deficiencies, and that it had a potential to be used as a treatment method for ADHD (Richardson & Puri, 2000). A

review conducted in 2006 by the same research group suggested that while current evidence does not support EFA supplementation as a primary treatment for ADHD, further research is warranted as omega-3 PUFAs may present an alternative treatment method in the future (Richardson, 2006).

Promising results have been found in open labelled studies looking at EFA treatment for ADHD (Germano et al., 2007; Joshi et al., 2006). Research found that a group of children with ADHD who were supplemented with flax seed oil (which is rich in ALA the precursor for EPA and DHA), demonstrated a significant improvement in the scores for inattention, impulsivity, restlessness and self-control after supplementation (Joshi et al., 2006). Likewise, another study found that a child population supplemented with omega-3 EPA for eight weeks showed an improvement in inattention and hyperactivity scores on the Conner's rating scale (Germano et al., 2007). While these results are promising, the experimental designs lack the robustness of a blinded study and are subject to more confounding variables. The validity of these results could be improved by replicating this research using other experimental designs such as double blinded placebo controlled randomised trials.

Milte et al. (2015) investigated the effects of EPA and DHA on attention, literacy and behaviour in children with ADHD. The study had 90 participants aged between 6 and 13, who were randomised to three different supplementation groups, an EPA-rich oil, a DHA-rich oil and a safflower oil formulation which acted as a placebo (Milte et al., 2015). The study adopted a 12-month randomised three-way cross-over design. While the outcome measures showed no difference between the three treatments, regression analyses revealed elevated omega-3 levels were significantly associated with improved literacy scores, attention and parent-rated measures of oppositional and hyperactive behaviour (Milte et al., 2015). Conversely, negative associations were detected between increased n-6 PUFA levels and various outcome variables (Milte et al., 2015). The authors note the limitations of the study, with the statistical power of the study being limited by the difficulties recruiting eligible participants and a 37% drop out rate. This was the first study to directly compare the effects of EPA and DHA supplementation in ADHD alongside blood sampling to investigate symptom outcomes with individual associations. It indicated that increasing DHA and EPA levels via supplementation may improve attention, literacy and behaviour in children with ADHD (Milte et al., 2015).

Gow et al. (2015) conducted a review, investigating evidence from meta-analyses, systematic reviews and clinical trials published over a 12-month period. The study found that the relative efficacy of EPA versus DHA remains unclear, but the body of research

that exists suggests that both play an important role. The authors suggest that due to its modest efficacy and relatively low risk, omega-3 supplementation could be a useful augmentation of traditional pharmacological interventions, and the further investigation of non-pharmacological interventions is warranted. (Gow et al., 2015).

When investigating the effect of EFA levels on ADHD, the direction of the relationship must be considered. While it has been found that EFA supplementation can be used as a modestly effective treatment for ADHD (Bloch & Qawasmi, 2011; Milte et al., 2015), it needs to be determined whether this indicates the ADHD is related to the low EFA levels, or if there is something else underlying the disorder. A meta-analysis conducted in 2011 showed the effect of omega-3 ADHDs in youths (Bloch & Qawasmi, 2011). In a sample of 699 children from 10 randomised, placebo-controlled clinical trials, results showed a small but significant effect in improving ADHD symptoms. All but one of the trials were a parallel design, with the study duration ranging from 7 weeks to 4 months. No significant relationship between trial duration and measured efficacy of supplementation was detected. The authors of this meta-analysis raised the question of adequate sample size. Bloch and Qawasmi (2011) stated to reach sufficient power to detect a moderate effect size of 0.31, the trials would need a population of around 330 children. As the sample sizes in the studies analysed ranged from 26 to 117, this suggests that the trials included in the meta-analysis are underpowered, and could contribute to the inconsistent pattern of results reported in the literature.

Despite all the research that has been done on the topic, at the present time there is no clear consensus regarding the efficacy of essential fatty acid supplementation as a treatment method for ADHD. Mixed results have been obtained when investigating this method and this may be due to methodological issues. The differing results may be attributable to the variety of treatments used in the studies. With differing levels of n-3, in particular variance in levels of EPA and DHA, it is not surprising that different studies are yielding different results. Also, studies are not all blinded, and the blinding procedures vary across studies. The evidence for the efficacy of EFAs as a treatment technique for ADHD is far from definitive, but there are not many options. There is public concern, often fuelled by the media, as to the safety of long term stimulant usage as a treatment method and this has attracted interest in looking for an alternative treatment method. EFA supplementation is an attractive option for this, as omega-3s has high safety and tolerability levels (Eritsland, 2000).

Due to this possible link between EFAs and ADHD symptomology, this thesis will investigate the link between the two. This will be answered in two parts, with the first

part investigating whether there is any difference between baseline EFA levels of people suffering from ADHD and healthy controls, and also whether EFA supplementation may alleviate symptoms of ADHD, produce changes in cognition or cardiovascular function, or neural function as measured by fMRI.

Chapter 5 Methodology

The current chapter will outline the tasks and techniques used in the experimental chapters of this thesis. It provides a comprehensive description of the experimental designs and the protocol of the studies. A detailed explanation of the sample used is given, describing the inclusion and exclusion criteria, and addressing sample size considerations. The pre-processing techniques used for the fMRI sections of the study will also be provided in this chapter, however specific analyses undertaken will be outlined in the relevant chapter.

5.1 Study Protocol

Ethical approval

Prior to the commencement of the trial, the experimental procedures of this thesis contained in the following chapters were ethically approved. Ethical approval was obtained from the Human Ethics Committee in the Faculty of Life and Social Sciences at Swinburne University, Melbourne Australia (SUHREC: approval number 2009/186). Participants provided written consent for their participation in this study, and a copy of the participant consent forms can be found in Appendix i: . Participants also received remuneration for their time and transport costs for being part of the study.

Experimental design

The first experimental chapter of this thesis (described in Chapter 6) looked at the effects of baseline levels of EFA on cognitive performance and cardiovascular performance in young adults both with and without ADHD. It used a cross-sectional design, focussing on the differences between control and ADHD participants on the variables measured.

The second study of the thesis adopted a clinical trial approach, utilising a placebo-controlled, double blinded, randomised parallel design. Participants were tested at baseline and after a 12-week supplementation period. Participants were randomly allocated to one of three treatment groups (placebo, EPA-rich or DHA- rich), with treatments detailed in section 5.3. Prior to the study, a computerised randomisation code was created to ensure both equal numbers of participants in each supplementation group and equal distribution of control and ADHD participants over the supplementation groups. Participants were assigned to a code number on the bottle according to the order in which they were tested, and whether they were an ADHD or a control participant. This code number remained consistent throughout the entire course of the study. The researchers remained blinded to which participant was on which treatment until the data collection for the study had ended.

5.2 Power calculation

Cognitive analyses

In order to gain sufficient power from analyses on cognitive data, the required sample size was calculated using previous research used to calculate sample size (B. H. Cohen & Lea, 2004). Cohen and colleagues categorised eta-squared values in small (.01), medium (.06) and large (.14) effect sizes, assuming a power value of 0.8 and an alpha criteria of 0.05 (B. H. Cohen & Lea, 2004). G*Power software 3.1.7 (Faul, Erdfelder, Lang, & Buchner, 2007) was used to calculate the required sample size for both the comparisons between ADHD and control participants and between supplementation groups. Using a medium effect size of 0.3, alpha of 0.05 and power of 0.95, it was determined that 111 participants were required for the study to have sufficient power to detect a moderate effect size. The total sample size of this study was 96, so it did not quite meet the criteria. It would have been statistically better to have a larger sample size, but due to the limitations of the study, 96 was deemed adequate.

For the comparison between supplementation groups, a repeated measures between factors ANOVA power calculation was conducted. Using an effect size of 0.4, alpha of 0.05 and power of 0.80, It was determined that 82 participants were required for the study to have sufficient power to detect a moderate effect size.

Cardiovascular analyses

The same effect size, alpha and power value was used for the cardiovascular analysis as the cognitive. The sample size required for both comparisons was the same as the sample size outlined above.

5.3 Procedure

During the first testing session held at Swinburne University, participants first read and signed an informed consent form, then completed some questionnaires, completing the DASS, Conner's ADHD rating scale and a demographic questionnaire. Questionnaires were administered in a quiet room and completed using pen and paper. Participants completed the questionnaires in their own time, with no time limits being imposed.

After completion of these questionnaires, participants completed the SUCCAB. At the second testing session, the Raven's advanced processing matrices were also completed.

Participants then underwent the cardiovascular testing, which comprised of blood pressure monitoring, transcranial Doppler and Sphygmocor arterial stiffness

measurements. At the completion of the session, the participants provided a finger prick blood sample for whole blood fatty analysis.

For participants who underwent an fMRI, they attended a second baseline session held at the Brain Research Institute which is located at the Austin Hospital campus in Melbourne. They underwent a screening process to ensure their safety, and a one hour scan. This involved a structural scan, and the Stroop task inside the scanner. A tabular representation of the methodology is presented below in Table 5-1.

Table 5-1: Testing protocol by session.

	Session 1a	Session 1b	Session 2a	Session 2b
Ethics	Informed consent form			
fMRI		Baseline fMRI (if fMRI participant)		After supplementation fMRI (if fMRI participant)
Questionnaires	Demographic and health questionnaire DASS Conners ADHD scale		Demographic and health questionnaire DASS Conners ADHD scale	
Cognitive tests	SUCCAB		SUCCAB Ravens Advanced Matrices	
Cardiovascular measures	Blood pressure Doppler ultrasound Spyhgmocor arterial stiffness		Blood pressure Doppler ultrasound Spyhgmocor arterial stiffness	
Blood measures	Finger prick whole blood sample		Finger prick whole blood sample	

Randomisation

As this study adopted a double blinded approach, both the researchers and the participants were unaware of which supplement each participant was taking. The student researchers were provided with numbered bottles which contained identical capsules. The supervisors, who were not part of the data collection process, received a list of bottle numbers and which bottle corresponded to group 1, 2 or 3. A random number generator in Excel then created a list of numbers to correspond with the sequence of supplementation allocation. The investigators were unblinded only once data collection and analysis were complete.

Supplementation

The study used three randomly assigned formulations, either an EPA-rich formulation, a DHA-rich formulation, or a placebo formulation primarily consisting of soya oil. All formulations were identical in appearance. Participants were instructed to take four capsules per day with food, and were advised not to consume more than the recommended dosage in order to avoid potential side effects of the capsules and to ensure all participants received the same dosage. All participants were provided with a supplementation checklist to remind them to take their supplements and to track their supplement intake. If the participants missed their supplementation for any reason, they were asked to keep the capsules for a final capsule count.

EPA and DHA were present in triglyceride form in both the EPA-rich and the DHA-rich formulations, and these represented 65% of the total of concentrated n3 fatty acid triglycerides. The remaining 35% consisted of partial glycerides. The EPA-rich formulation had an EPA to DHA ratio of 4.5:1 and the DHA rich had an EPA to DHA ratio of 1:4. Additionally, both the EPA-rich and the DHA-rich formulations contained 4mg of d-alpha-tocopherol, which has anti-oxidant properties, and 4.4 mg of betacarotene, which was used as a colouring agent and also has anti-oxidant properties. The capsule shell for both formulations was made up of 183mg of gelatine, 87 mg of glycerol and 21mg of purified water per capsule.

Table 5-3 below shows the essential fatty acid composition of the placebo treatments used in the study. The placebo treatment consisted of 500mg of soya bean oil, and 2.2mg of beta-carotene. The capsule shell was composed of 120mg of gelatin, 55mg of glycerol and 25mg of purified water per capsule.

Table 5-2: Daily amount (mg) of EPA and DHA provided by dosage of supplementation.

Omega-3 Supplementation	EPA	DHA
EPA-rich	1100	240
DHA-rich	280	1140

Table 5-3: Fatty acid composition of placebo capsules.

Placebo	Composition of Fatty Acids (%)
Linoleic acid (18:2 n6)	53
Alpha Linolenic acid (18:3 n3)	8
Oleic acid (18:1 n9)	23.5
Palmitic acid(16:0)	11
Stearic acid (18:0)	4
Arachidic acid (20:0)	1
Eicosenoic acid (20:1)	1
Behenic acid (22:0)	1
Palmitoleic acid (16:1)	0.3
Myristic acid (14:0)	0.2

5.4 Inclusion and exclusion criteria

The participant pool was the same in all experiments of the thesis. The inclusion criteria varied between control and ADHD participants, and the criteria for both groups are outlined below.

Control participants

Inclusion criteria for control participants were carefully monitored. Participants were between 18 and 40 years of age. There are two main reasons this age range was selected. Firstly, there is little research conducted on ADHD in young adults, and this thesis aimed to add to the literature. Hence, in order to get a matching control sample, the controls had

to be within the same age range. Secondly, cognitive performance and cardiovascular functioning have been found to decline with age, and by testing people at a reasonably young age it is hoped that the variability in performance levels due to age can be minimised. Participants also had to be in general good health, with no current significant general health issues.

Control participants were excluded if they had a history of psychiatric disorders. This was because some research has suggested that essential fatty acids may have some potential benefit in psychiatric disorders such as major depressive disorder and bipolar disorder (Freeman et al., 2006). Additionally, people with certain psychiatric disorders may metabolise fatty acids differently to the general population, with depression being associated with lower omega-3 levels in red blood cell membranes (Maes et al., 1999). Participants with a history of epilepsy were excluded, as some of the tasks involve flickering stimuli on a screen, which may trigger seizures in participants who suffer from this disorder.

Participants were also excluded from the study if they were already taking fish oil supplementation, or if they had taken fish oil supplementation in the three months before commencing the study. This is because if the participant had taken fish oil supplementation in the preceding three months, any effects detected may be due to the influence of this fish oil rather than the supplementation given for the study.

Participants were excluded if they had a history of cardiovascular disease. This was because one of the variables being examined in this thesis was cardiovascular performance. If a participant had a history of cardiovascular disease, then this may cause differences in baseline reading compared to that of other participants. Therefore, only participants without a history of cardiovascular disease were recruited to ensure the maximum probability that any differences in cardiovascular performance were due to the effect of the supplementation and subsequent changes in essential fatty acid level, and not due to any differences caused by cardiovascular problems. This minimises the variability in cardiovascular performance between participants.

Similarly, participants did not have a history of neurological disorders. This was because the study is investigating the effects of essential fatty acids on the brain, and if participants have any brain abnormalities or neurological disorders then it may have an effect on the results.

Control participants were not on any prescription medication, with the exception of the birth control pill. This was because any medication may interact with the effect of the fish oil and affect the results of the trial.

ADHD participants

The inclusion criteria for the participants with ADHD were overall similar to those for the Controls. Researchers initially set out to recruit a population with a clinical diagnosis of ADHD. This however presented some problems, as many participants were unwilling or unable to obtain a clinical diagnosis from their clinician, largely due to the time separation between (child) diagnosis and (adult) recording. The sample used was one that exhibited symptoms of ADHD and self-identified in this way. In order to assay the level of ADHD symptoms at the time of experimentation, the scores on the Conner's Adult ADHD rating scale were analysed for significant differences between the ADHD and control groups.

The participants with ADHD did suffer from a psychiatric disorder in ADHD. Additionally, some of these participants had a history of depression and anxiety disorders. This is to be expected, as it is very common for sufferers to experience a co-morbid disorder, with as many as 60% of sufferers of adult ADHD experiencing an co-morbid condition at some time in their life (Spencer et al., 1996). However, at the time of testing ADHD participants had no current co-morbid disorders, and if they did fulfil the criteria for another disorder in the past, the primary diagnosis was ADHD. It would have been ideal to recruit a population of participants with no co-morbid conditions, but this was not a practical population to recruit. While it would have been ideal to administer a clinical interview, such as DIVA, the research team was not qualified to conduct this assessment.

Additionally, a large section of the ADHD population was on medication for their condition throughout the trial. Their medication remained stable throughout the trial, determined by none of the participants changing prescriptions during the supplementation period. In any clinical trial, it is ideal to have participants on no medication, so that the independent variable (in this case the type of fish oil) is the only difference between the participants. Unfortunately, as there were issues with recruitment, it was almost impossible to obtain a sample that were not currently taking stimulant medication so the current sample was obtained. It would also have been better to have the participants withdraw from their medication for the duration but this would not have met ethical guidelines and could have been detrimental to the well-being of the participants, and possibly dangerous. Also, the confound of actually measuring the effects of withdrawal of medication would have to be accounted for. While records of medication

were collected, the information was not recorded in a way that was able to be codified in the thesis in a reliable way.

5.5 Participant Recruitment

Participants were recruited through posters, newspaper advertisements, the internet and general word of mouth. Advertisements were placed around the Swinburne University of Technology campus, on various internet sites and in newspapers relevant to the target population. Additionally, the researchers contacted professionals who treat ADHD in order to aid recruitment. The researchers held a meeting with these practitioners to discuss the research. They explained the concept of the study, placed posters in the clinic, and asked the clinicians to mention the study to their patients.

On these advertisements, a short statement on the purpose of the research was listed, alongside the exclusion criteria. The advertisements invited participants to contact the researchers by phone or email if they were interested to participate.

Once people contacted the researchers, the potential participants were then screened to see if they met the eligibility criteria. If they were interested, and met the criteria, then a time was set up for the first testing session. All participants were asked if they were interested in the fMRI.

5.6 Data screening, analysis and processing

Cognitive screening

Prior to any statistical analyses being conducted the SUCCAB data was screened for missing data, out of range values and outliers. The first stage of this process was to eliminate all reaction times less than 150 milliseconds and recode these as missing. This was undertaken as previous research has determined anything less than 150ms as too rapid for a valid response, and hence has utilised 150ms as a minimum valid response time (Hultsch, MacDonald, & Dixon, 2002). This avoided the issue of recording delayed responses to previous stimuli. Additionally, all accuracy vales of less than 50 percent, along with their corresponding response times, were also recoded as missing. Results such as these were considered to reflect a lack of understanding of the task, and therefore invalid responses. After this initial screening process, histograms and box plots were then used to screen for outliers. Any values that were more than two standard deviations from the mean were recoded as missing values along with the corresponding reaction time or accuracy.

As recommended by Tabachnick and Fidell (2007) , it was then checked that all cases missing more than 30% of data were removed from the dataset. This was done to ensure

any participants who had a consistent problem with the SUCCAB tasks, whether this be through lack of understanding or technical issues, did not affect the data of the group as a whole.

All of the computerised cognitive tasks were undertaken in the same room under the supervision of a researcher. This was selected as it allowed for millisecond precision for the stimulus presentation, to ensure synchronisation with the screen refresh signal. For all of the computerised cognitive tasks, with the exception of SUCCAB delayed recognition memory, participants had a practice run through of the task. This was so they could familiarise themselves with the task before the experiment began.

5.7 Tasks

SUCCAB

A version of the Swinburne University Computerised Cognitive Ageing Battery (SUCCAB) was utilised in this study to assess cognitive ability. This battery was chosen due to its sensitivity to changes as a result of nutritional interventions, such as flavonoids, vitamin C and multivitamins(MacPherson, Ellis, Sali, & Pipingas, 2012; Pipingas et al., 2008). The SUCCAB has been used in previous studies as a measure of cognitive performance (MacPherson et al., 2012; Pipingas et al., 2008). This study used the following tasks from the battery, simple reaction time, complex reaction time, immediate recognition memory, spatial working memory and delayed visual memory. The SUCCAB tasks were presented on a 17-inch CRT monitor and used a DOS-based operating system.

Each participant was given a handheld button box to respond to the SUCCAB battery, with four buttons. The buttons were laid out in a compass configuration (top, bottom, left and right) and were four different colours (red, green, yellow and blue). Depending on the task, the participant pressed different buttons either due to the location of the button or the colour of the button. Alternate versions of the SUCCAB were used each testing session so there was no chance of interference between the sessions. Scores for each task were recorded as mean reaction times and percentage of accurate responses. Therefore, higher reaction times scores reflected slower mean reaction times and higher accuracy reflects more accurate scores.

Simple reaction time was measured in order to measure cognitive processing time, and motor response speed. Participants were presented with a white square on the screen, and were asked to press the button on the right as soon as they saw the white square. A random time interval followed the appearance of each stimulus to avoid anticipation effects.

Complex reaction time was measured in order to measure visual perceptual processing time and motor response speed when participants were faced with a forced choice of two options. Participants were presented with either a red square or a blue triangle and utilised the red (right) and blue (left) buttons on their gaming pads to respond to the corresponding shape. Participants were asked to press the corresponding coloured button as quickly as possible.

Immediate recognition memory was tested using visual stimuli. A series of 40 abstract images were presented in the centre of the screen. Participants were instructed to try to remember these images, as they would be asked to recall them. Immediately afterwards, they were presented with another series of 40 images, 20 of which they had seen in the original presentation and 20 which were new. Using the left or right button, participants indicated whether they had seen the image in the first presentation or whether the image was new. This task is a measure of non-verbal recognition memory.

To measure spatial working memory, participants' ability to hold a pattern in their mind was tested. Participants were presented with a white 16 square (4X4) grid on a black background. For each trial, six of the grid references were filled in by a white square. This pattern was displayed for two seconds. After this, participants were presented with an empty grid, where one square was filled at a time. Using the yes and no (left and right) options on the gaming pad, participants indicated whether these positions matched the initial locations in the grid.

Delayed recognition memory was tested using a similar method to immediate recognition memory. This was used to see how well the memory functions after a longer delay. At the end of the testing session, approximately 30 minutes after originally seeing the images, participants were presented with another series of 40 images. 20 of these images had been presented in the simple recognition memory task, and 20 of the images were new. Participants pushed either the left or right button, depending on whether they had seen the image in the simple recognition memory task.

Stroop Task

The Swinburne Stroop task was an adaptation of the Delis-Kaplan Executive Function System(D-KEFS) Color-Word Interference task. This task was programmed specifically for the study using E-Prime software. This task was composed of three conditions, a congruent condition, an incongruent condition, and an advanced congruent condition. In each of the conditions, which are detailed below, the participants were instructed to respond with a press of one of four corresponding buttons. All stimuli appeared for 1000

milliseconds, followed by a fixation cross that was presented for 2000 milliseconds. The stimuli were broken into blocks, with two blocks of each stimuli presented. Each congruent block contained 12 stimuli, and each incongruent block and advanced incongruent block contained 16 stimuli. This totalled to 24 congruent stimuli and 36 incongruent and advanced incongruent stimuli being presented. Accuracy, measured by percentage of correct responses, and mean reaction times, measured in milliseconds, were recorded for each response.

In the congruent condition, the words red, blue, green and yellow were presented in the matching text colour with no interference. The participant was asked to push the button that corresponded to the colour of the text as quickly as possible.

In the incongruent condition, the same words were presented in red, blue, green or yellow text, but in this condition the colour of the text did not match the written word. For example, the word "Red" was written in blue, green or yellow coloured font. In both the congruent and the incongruent conditions, the participant's task was to push the corresponding coloured button for the colour of the text, not the written word.

In the advanced incongruent condition, all of the stimuli words were presented in incongruent colours. Some of the stimuli were surrounded by a black rectangle. If the stimulus was contained inside a black rectangle, the participant was asked to press the key corresponding to the meaning of the word. If the word was presented independently of the rectangle, then the participant was asked to press the button that corresponds with the colour of the text.

5.8 Questionnaires

Health and Demographic Questionnaire

Participants completed a health and demographic questionnaire (see Appendix ii:). This questionnaire was created by the researchers specifically for the purpose of this study, and collected information such as height, weight, age and education level. It also investigated aspects of health, including the frequency of exercise, and whether they were on any medication or health supplements. The questionnaire changed slightly for the second testing session, with the questionnaire asking if there had been any significant changes in the lifestyle of the participant during the supplementation period, in diet, exercise, sleeping patterns or any other lifestyle factor. This was so that if any major health or lifestyle changes did occur during the supplementation period, then researchers could recognise this and determine whether it may have affected the participants' performance over and above the effects of the supplementation.

Depression Anxiety Stress Scale

In order to measure depressive symptoms before and after supplementation, participants completed the Depression Anxiety Stress Scale (DASS) (Lovibond & Lovibond, 1995). They completed this scale twice, at baseline and following the 12-week supplementation period. This measure was collected to measure the psychological state of the participants, to see if it changed over the supplementation period, and to see if any differences were present between the ADHD and the control participants.

The DASS is composed of 42 self-reported items, which are designed to measure the emotional states of depression, anxiety and stress, and determine their individual effect on the participant. Each item on the questionnaire is categorised into one of these three dimensions, with there being 14 items in each category. Scores for each of the respective categories are calculated by adding the scores for the respective items.

The 14 items that form the depression subscale of the DASS measure symptoms including dysphoria, hopelessness, lack of interest and self-depreciation. The items that form the anxiety subscale of the DASS focus on the symptoms of anxiety, including autonomic arousal, situational anxiety, physical symptoms of anxiety and the participants' subjective experiences of anxiety. The items that make up the stress subscale of the measure assess the ability of the subject to relax, the level of chronic stressful arousal, and their general patience and irritability levels. While these three dimensions are conceptually different, there is some clinical overlap between them (Lovibond & Lovibond, 1995).

Participants read each item carefully, and circled the corresponding number as to how they have felt in the past week. They were informed there were no right or wrong answers, and to not spend too much time on any statement. Responses were measured on a 4-point Likert scale, ranging from 0 ("Did not apply to me at all") to 3 ("Applied to me very much, or most of the time"). The score for each item was totalled along with the other items in its respective category, and a score from 0-42 was calculated, with 0 indicating a satisfactory emotional state and 42 indicating severe emotional distress.

Norms created by Lovibond were used to categorise the severity of each dimension as normal, mild, moderate or severe (Lovibond & Lovibond, 1995). This measure was chosen as it has been found to have satisfactory psychometric properties, and has been found to correlate with other measures designed to measure similar concepts, such as the Beck Depression Inventory and the Beck Anxiety Inventory (Lovibond & Lovibond, 1995). It has also been found to have high validity and test-retest reliability (J. R. Crawford & Henry, 2003).

Conners Scale

The Conner's Adult ADHD Ratings scales were used to measure the presence and severity of ADHD symptoms of participants in the study. This measure provides a multiple-informant assessment of the symptoms with self-report (CAARS-S:L) and observer rated scales (CAARS-S:L). The observers were significant people in the participants lives, such as partners, parents, friends or siblings. These observers were selected by the participants themselves. By utilising the multiple informant method, it increases reliability when symptoms are often difficult to measure objectively. The long versions of these scales were used to collect detailed information. Identical scales are used for both the participant and the observer forms, and from these there are also identical subscales and indices.

The Conner's scale is composed of 66 items, which are designed to measure the presence and severity of ADHD symptoms. There are eight different subcategories categorised by their symptoms. The eight categories are inattention and memory problems, hyperactivity and restlessness, impulsivity and emotional ability, problems with self-concept, DSM-IV inattentive symptoms, DSM-IV hyperactive-impulsive symptoms, DSM-IV ADHD symptoms total and ADHD index. Scores for each of the respective categories are calculated by adding the scores for the respective items. Each item on the questionnaire is categorised into one of these dimensions, with there being between 6 and 12 items in each dimension. The eighth dimension is calculated by adding the scores for the sixth and seventh dimension together. Each item was measured on a 4-point Likert scale, ranging from 0 ("Not at all, never") to 3 ("Very much, very frequently"). An inconsistency index is also calculated in order to detect contradictions within answers. If the total of this is 8 or greater, the authors recommend the results be interpreted with caution.

Ravens Advanced Matrices

Participants also completed a modified version of the two sets of Raven's Advanced Progressive Matrices test. This test is used to estimate the intellectual abilities of the participants and to determine if any differences were present between groups. One of the advantages of this test is that the stimuli are visual, and do not rely on the verbal skills of the participants. This is useful when the sample group is culturally diverse, and English is not always the participant's first language, or if the participants have difficulty with reading and writing.

The test administered ran for 20 minutes. While the traditional version of this test is 40 minutes, this would have extended the testing session to be very lengthy, and this may

have reduced compliance, particularly in the population suffering from ADHD. Research has found a strong correlation between scores on the short and long versions of the task ((Hamel & Schmittmann, 2006), so researchers deemed it appropriate to use the shorter version.

This test was administered on the second testing session of the study, in order to shorten the duration of the first testing session. This test is composed of 48 items, which become progressively harder. Each item is composed of an incomplete matrix, and eight options are presented below. One of the options presented fits into the matrix and the participant is asked to identify which of the segments fits into the matrix. They were asked to complete as many of these as possible in 20 minutes. Participants were given a score out of 48, with correct responses scoring one point, and wrong or no responses scoring zero points. No points were deducted for incorrect answers.

5.9 Physiological Measures

Cardiovascular Measures

Cardiovascular function was measured in three different ways. It was measured through blood pressure, through cerebral blood velocity through the carotid artery, and through arterial stiffness measures.

Blood Pressure

Blood pressure was measured using an automated sphygmomanometer designed to measure blood pressure for the brachial artery. The participant sat down and relaxed for two minutes to ensure a resting blood pressure reading was obtained. Participants were asked to remain silent, relax and stay still throughout the assessment. The cuff was placed on the participants left arm and the sphygmomanometer was activated. The blood pressure of the participant was then recorded.

SphygmoCor

Arterial stiffness was measured using a SphygmoCor pulse wave form analyser (SCOR; PWV Medical, Sydney Australia). This apparatus is a non-invasive pencil-like device which measures the ascending aortic waveform from the radial artery (Pase et al., 2010). This device calculated several indicators of arterial stiffness including the augmentation index, the augmented pressure and blood pressure data. This augmentation index is an indirect measure of arterial stiffness, which has been associated with cardiovascular disease (Cohn, 2006; Lam et al., 2009; Mattace-Raso et al., 2006). The operator index is an indication of the quality of the signal, ranging from 0-100. According to the

manufacturer's instructions, if it is 80 or above, the recording should be accepted, and if it is below 80 procedures should be repeated in order to gain an adequate recording.

The participant's height and weight and blood pressure were recorded and entered into the devices software prior to the assessment. The participant sat down in a chair in a relaxed position with their left arm on the table with their palm facing upwards. They rested their left arm on a small arm rest in order to maintain stillness during the testing. In order to detect radial pulse, the tonometer was placed perpendicular to the participant's wrist. The researcher located the strongest pulse, and placed the tonometer on it to record a consistent and accurate waveform. When a suitable waveform was detected the recording was saved. Only recordings with operator indices 80 or above were included to ensure the reliability of the data collected. The procedure for this aspect of the study was based on the recommended standard conditions for the measurement of arterial stiffness (Laurent et al., 2006).

Another measure of cardiovascular health recorded was the transcranial Doppler ultrasound. This ultrasound device measures the blood velocity to the brain and this technique has been found to have clinical relevance (Holdsworth, Norley, Frayne, Steinman, & Rutt, 1999; Lindegaard et al., 1987).

The Doppler measurements were taken directly after the Sphygmocor readings. The participant sat down in a chair and was given a brief background on what the recordings were measuring. The computer set the probe at 4mz, in order to measure the common carotid artery. Water soluble gel was placed on the probe. The probe was placed on the participant's neck and gently moved around until a good signal was detected. This signal was then recorded for approximately one minute and saved. The probe was then reset at 2mz to measure the middle cerebral artery. The probe was then placed on the side of the skull, near the participant's ear. The depth and gain were adjusted until a strong signal was detected, and this signal was then recorded for approximately one minute.

The cardiovascular data was then screened to remove any missing data, out of range values and outliers. Due to difficulty in obtaining a clear MCA Doppler signal, there were several participants with missing MCA data. The researchers searched the literature for an adequate method to replace the missing MCA data with, but this was not obtained. It was noted that a large amount of MCA Doppler data was missing because of difficulties in obtaining a reliable and valid signal. As a result of this, the MCA data was removed from further analysis. As the majority of the missing MCA values were due to the inability of the researcher to locate an adequate signal from the MCA, the corresponding CC recordings were kept for analysis.

Blood Tests

The blood measurement chosen for this study measured whole blood fatty acid levels. This was measured using the HS-Omega-3 index, a high sensitivity omega-3 index finger prick blood test (W. Harris & C. von Schacky, 2004). This index is calculated as the sum of EPA and DHA levels in the blood and has been shown to be negatively correlated with cardiovascular disease risk (W. Harris & C. von Schacky, 2004). This method has been established through research, and has been found to have predictive power for cardiovascular disease (Shearer, Pottala, Spertus, & Harris, 2009). The methodology of the HS-omega 3 index is a standardised unit of measurement, and has demonstrated reliability in terms of constancy checks and quality measurement (Harris & Thomas, 2010).

There are many reasons why this method was chosen over traditional venous blood tests. Firstly, some participants were uncomfortable with the idea of the needle associated with venous blood tests. The researchers felt that this may affect compliance with the blood testing protocol. As the appropriate sample was already difficult to obtain, researchers wanted to maximise the number of potential participants, and felt this would be easier to achieve with a finger prick method. Similarly, the student researchers can undertake the finger prick method, as no medical training is necessary to collect the blood. This means that the blood can easily be collected at the time of testing and there is no need for the researchers to employ a nurse, or for the participants to visit a pathology clinic which may increase the risk of poor compliance.

The participant sat down in a comfortable position. The researcher took the index finger of the participant's non-preferred hand, and cleaned it with an alcohol swab. The alcohol was then wiped from the finger with gauze, and the finger left to dry for one minute. The finger was then pierced with a sterile, one use lancet device, and a drop of blood formed. The initial drop of blood was wiped away with a tissue in case the alcohol wipe had contaminated it. The next droplet then formed until it was big enough to fall as a drop (approximately 0.2 ml of blood) and was collected on filter paper. This was done by gently squeezing the participant's finger until a droplet formed, then carefully lowering the paper to touch the drop without the fingertip touching the paper. When the blood on the paper filled one of the small circles, pressure was placed on the fingertip with a tissue to stop the bleeding and a band aid was applied. The filter paper was left to dry for 15 minutes, then placed in a snap lock bag containing desiccant crystals, labelled and placed in a -80 degree freezer for storage.

Once data collection was complete, the samples were flown via courier to the Omega Metrix blood pathology laboratory in Germany, where the samples were analysed using capillary gas chromatography (Harris & Von Schacky, 2004). The results were then sent via secure email to the researchers once the analysis had taken place.

5.10 Functional Magnetic Resonance Imaging

Imaging procedures

Participants who took part in the fMRI part of the study each underwent two scans, one at baseline and one after the 12-week supplementation period. Scans were conducted at the Brain Research Institute in Heidelberg, Melbourne, Australia and were conducted using a 3 Tesla Siemens Tim Trio MRI scanner (Siemens, Erlangen, Germany) which was fitted with a 12-channel head coil. At the beginning of the baseline session, a high resolution T18 weighted image was acquired (coronal slice acquisition). This used a 3D MPRAGE sequence (TR = 1900 ms, TE = 2.6 ms, 192 slices, 0.9x0.9x0.9 voxels, FOV 230 mm, slice thickness 0.9 mm). For the testing session conducted after supplementation, 66 functional images were obtained using a T2 weighted gradient echo-planar pulse sequence (TR = 3000, TE = 30 ms, FOV = 216 mm, voxel size 3 x 3 x 3 mm).

Upon arrival at the scanning facility, each participant undertook a safety screening procedure. This was in addition to the phone screening procedure conducted at time of recruitment and was conducted by a qualified radiologist. After this, the participant removed all metal (jewellery, electronic devices etc.) from their body and entered the scanner in a supine position. For the duration of the scan, participants were asked to stay as still as possible in order to minimise head movement. Foam padding was placed around the participant's neck to assist this. During scanning the participant was in contact with the researchers and the MRI technician via a MRI-compatible microphone. Stimuli for the task were presented on a MRI-compatible screen located behind the scanner, viewed by the participants with the assistance of a mirror.

Imaging analyses

Pre-processing and statistical analyses were performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK). Before pre-processing commenced, the first six volumes were discarded from each functional sequence to reduce T1 saturation effects in image time-series. The "ArtRepair" tool was then used to clean voxels and repair slices with high variance levels. These corrected images were realigned to the first image of the first session creating a mean realigned image. The T1-weighted structural image was then co-registered to the mean re-aligned image that has been created. This was visually

checked and spatially normalised to the template provided by SPM8. This T1 spatial normalisation was applied to the realigned EPI images and these were smoothed through the use of a Gaussian kernel. ArtRepair was then used to detect and repair any volumes which still exhibited high variation in signal intensity.

5.11 Conclusion of Chapter

This chapter described the methodology, tasks and techniques which are used in the experimental chapters of this thesis. Methodologies that are specific to an individual chapter will be described in the methodology section of the relevant chapters.

Chapter 6 Baseline results

6.1 Abstract

In this chapter, baseline differences between control and ADHD participants are investigated. The present chapter aimed to investigate the baseline differences in demographic, EFA levels, cognitive performance and cardiovascular function. The control and ADHD participants were comparable in demographic and EFA blood level variables. As predicted, a significant difference in ADHD symptoms between the control and ADHD participants was detected. Results from this chapter reveal that results showed that there were no significant differences in the majority of cognitive tasks between the ADHD and control participants at baseline. Similarly, there were no significant differences in the majority of cardiovascular functions tested between the groups at baseline. These results provide the basis for the experimental chapters to follow.

6.2 Introduction

In combination with an active lifestyle, a balanced diet is an important factor in the health and wellbeing of humans. Research has shown that nutritional intake can impact on many areas of health, including cognitive function (Fontani et al., 2005) and cardiovascular performance (S. Connor & Connor, 1997; W. E. Connor, 2000). As nutritional intake through diet is an important aspect of human health, research into such nutrients and how they interact within the body can provide numerous benefits. An important part of a balanced diet is the inclusion of adequate sources of essential fatty acids. As has been highlighted in the previous sections of this thesis, previous research has suggested that reduced levels n-3 PUFAs play an important role in many aspects of health, including but not limited to cardiovascular disease (Bucher et al., 2002; De Lorgeril et al., 1994), cognitive performance and neurodevelopmental disorders such as ADHD (Richardson & Puri, 2000; Stevens et al., 1995). Given this, it is likely that n-3 PUFAs play an important role right throughout the lifespan.

Although the agricultural revolution marked the beginning of the dramatic change of dietary intake in humans, it is only since the 1990's that there has been a significant increase in the occurrence of lifestyle diseases such as obesity (Mokdad et al., 1999) and diabetes (Mokdad, 2000). Research has implied that the co-morbidity of the diseases is significant, with both high body mass index (BMI) and weight gain being major risk factors for diabetes (Mokdad, 2000). In order to avoid these dietary changes becoming problematic down the track, preventative strategies could be implemented to solve the dietary problems before the consequences fully eventuate. As nutrition has been found to

be an important factor in these disorders, improving the dietary intake of the population using knowledge gained through research may lower the prevalence of these diseases in the future.

As discussed in depth earlier in the thesis, essential fatty acid status has been linked to cognition (Fontani et al., 2005; Kidd, 2007). Research has suggested that higher essential fatty acid levels play an important role in the brain function of all ages, ranging from infants to the elderly (J. Cohen et al., 2005; Fontani et al., 2005; Kidd, 2007; Uauy & Dangour, 2006). Sufficient amounts of DHA in the diet have been shown to aid brain development, improve learning ability, and have a positive effect on general cognitive functioning (Horrocks & Yeo, 1999).

In addition to the large amount of support for the effect of essential fatty acids on cognitive function, there is still conflicting evidence on this topic. As discussed earlier in the thesis, while many studies suggest that essential fatty acids do have an effect on cognitive performance (J. Cohen et al., 2005; Fontani et al., 2005; Kidd, 2007; Uauy & Dangour, 2006), other studies have found no evidence essential fatty acids on cognition (Rogers et al., 2008). Although Assisi (2008) does suggest that reduced levels of n-3 long-chain polyunsaturated fatty acids are associated with mental disorders of the central nervous system, the research failed to find any significant effects of supplementation in these patients. This brings up the issue of publication bias, which occurs when investigators, reviewers and editors tend to submit or accept studies for publication based on the direction or strength of the findings (Dickersin, 1990).A large body of research has reported bias in clinical trial research (K. Lee, Bacchetti, & Sim, 2008; Turner, Matthews, Linardatos, Tell, & Rosenthal, 2008) and this phenomenon is not exclusive to independent research projects, with research showing it is also present in meta-analyses (Kicinski, Springate, & Kontopantelis, 2015). A recent investigation into publication bias in meta-analyses from the Cochrane database found that it is an issue, with Bayesian analysis suggesting outcomes favouring treatment were 27% more likely to be included in meta-analyses (Kicinski et al., 2015). In order to counteract this bias, many prominent journals have stated that research sponsored by pharmaceutical companies cannot be published unless it is registered on a public database prior to beginning the trial. This was to increase the validity of the results published and to encourage the publication of non-significant findings.

Essential fatty acid status has also been associated with cardiovascular performance as discussed in Chapter 3(Frenoux, Prost, Belleville, & Prost, 2001; Wijendran, 2004). In both epidemiological and clinical research, results have indicated that higher levels of

EFAs are associated with healthier cardiovascular systems. Epidemiological research has found that essential fatty acid status may act as inhibitory mechanism against cardiovascular disease (Bang et al., 1980), and clinical research has supported the idea that a relationship exists between EFA status and cardiovascular health (Bucher et al., 2002)

While the issues arising from low EFA levels are present in the general population, these differences are also present in a population who present with symptoms of ADHD. Previous research has shown that PUFA levels have been implicated in certain symptoms of ADHD (Sinn & Bryan, 2007), further investigation of this relationship is warranted. Many past research studies have indicated a relationship between the symptoms of ADHD and lower levels of EFAs (Burgess et al., 2000; Mitchell et al., 1987; Stevens et al., 1996). In turn, it could be seen that this may lead to a link between cognition and ADHD. If EFA status is related to cognitive performance and people with ADHD have lower levels of EFA than the general population, it is possible that there may be differences in cognitive performance between the groups as a result.

It is important to note that while there is a large amount of research in the area, almost all of the research into ADHD has been conducted on children. Whilst ADHD is synonymous as a childhood developmental disorder, there is evidence that ADHD presentations continue into adulthood. Similarly, a childhood history of ADHD is currently necessary to obtain the adult form of the diagnosis (American Psychiatric Association, 2013b). Thus, limited research regarding the adult form of forms of ADHD has been published. As a result of the limited amount of research conducted in the area, the relationship between baseline n-3 PUFA levels, cognition and cardiovascular performance in a young, healthy population remains largely unknown. In addition, if such a relationship does exist, it remains to be established how the presence of ADHD symptoms may affect this relationship.

It can be seen from the previous research above that EFA status can have wide ranging effects on the human body. Although baseline results cannot measure causal factors, it is important to measure participants at a baseline level to gain an insight into the levels of these acids in the target population. Results from baseline studies can give an insight into where future research can go, and where to direct this research. Additionally, it is useful to allow researchers to see the differences between ADHD and control participants. Finally, baseline data provide the springboard for causal investigations into the effects of altered PUFA intake

6.3 Aims

The aim of the present study was to investigate the difference in baseline EFA levels between participants with and without ADHD. In particular, the study aims to investigate the relationship between baseline essential fatty acid levels and cognitive performance and cardiovascular function in a young adult population.

6.4 Hypotheses

As participants were matched, no differences were predicted between control and ADHD participants on demographic variables. It was predicted that ADHD participants would score higher on the Conners ADHD rating scale than the controls both on the self-report scale and the observer scale. Based on previous research which suggests that ADHD has a high comorbidity rate with other disorders (Gillberg, 1998; Richardson, 2006), it was predicted that ADHD participants would score higher on the subscales of depression, anxiety and stress than the control participants.

Multiple studies on people who suffer from ADHD have found that they have low levels of essential fatty acids in comparison with age and gender matched controls (Burgess et al., 2000; Stevens et al., 1996). While the majority of this previous research was conducted on children, it stands to reason that these findings may be replicated if the same research was conducted on adults. Therefore, it was predicted that those participants who suffer from ADHD would have lower levels of essential fatty acids (all of DHA, EPA, ALA, LA) compared with control participants.

Similarly, it was also predicted that participants with ADHD would have a lower level of cardiovascular performance than the control participants. This is based on the earlier prediction that ADHD participants would have lower level of EFAs than control participants. If cardiovascular performance is related to EFA status as suggested in section 3.2, it stands to reason that there may be differences between control and ADHD in cardiovascular performance.

Based on previous research (Lunn & Theobald, 2006), it was hypothesised that participants with higher levels of omega-3 PUFAs would perform better on the cognitive tasks than those participants with a lower level of omega-3 PUFAs. Better cognitive performance will be demonstrated by faster reaction times and higher accuracy in the SUCCAB battery and Stroop tasks. It was also predicted that participants with high levels of n-6 fatty acids, both as individual acids and relative to n-3, would have slower reaction times and lower accuracy on cognitive tasks. More specifically, it was predicted that the particular cognitive task that would be most affected would be spatial working memory,

as previous research has found it to be the most sensitive of the tasks in the SUCCAB battery (Pipingas et al., 2008).

Additionally, it was predicted that participants with ADHD would have a poorer level of performance on the cognitive tasks than control participants. This is based on the earlier prediction that ADHD participants would have lower level of EFAs than control participants, and as EFA status is related to cognition as shown in section 3.2, then it is expected that they will exhibit lower levels of cognitive performance.

6.5 Method

A full description of the methods used in this study can be found in chapter 5. The following methods section provides a summary of the methodology used in this experiment.

Participants

Ninety-six participants were tested in this study, consisting of 59 males and 37 females, with a mean age of 24.81 years (SD=4.84) with ages ranging from 18 to 40 years. All participants lived in Australia and had completed secondary school education. For inclusion criteria, exclusion criteria and recruitment methods, see section 5.4. All participants signed an informed consent form, in accordance with the Swinburne University Human Research Ethics Committee.

Procedure

Participants attended a Swinburne University testing laboratory session at a time convenient to them. They read and signed an informed consent form then completed the depression anxiety stress scales (DASS), Conners ADHD rating scale, and a demographic questionnaire. Questionnaires were administered in a quiet room and completed using pen and paper. Participants completed the questionnaires in their own time, with no time limits being imposed.

After completion of these questionnaires, participants completed the cognitive suite of tasks, including the SUCCAB and the Swinburne colour Stroop task.

6.6 Results

Demographic results

In order to analyse the differences in demographic variables between control participants and ADHD participants at baseline, an independent groups t-test was conducted. Participant demographics for the control participants and the participants with ADHD are shown in Table 6-1.

Table 6-1: Demographic data by Control/ADHD.

	Control	ADHD	t	p
Number of participants	60	38		
Gender (Males %/Females %)	66/33	50/50	-1.65	.103
Age	24.80 (4.94)	24.97 (4.76)	-1.72	.864
Height (cm)	173.47 (10.47)	172.14 (10.65)	0.58	.565
Weight (kg)	71.72 (13.48)	70.92 (13.82)	0.27	.787
Body Mass Index (BMI)	23.79 (3.52)	23.87 (4.36)	-0.89	.929
Education Level[1]	3.42 (1.08)	2.91 (1.04)	2.22	.029*
Ravens score	27.02 (6.27)	27.20 (6.88)	-1.20	.905
Exercise[2]	3.60 (1.03)	3.53 (1.40)	0.47	.794
Smoking status[3]	1.62 (0.64)	1.34 (0.59)	1.09	.041*
Fish Intake[4]	2.48 (0.99)	2.39 (0.87)	2.09	.640
Fruit and vegetable intake[5]	1.95 (.87)	2.11 (0.92)	-0.86	.393
Junk food intake[6]	3.20 (0.76)	3.33 (0.83)	-0.81	.421
Takeaway intake[7]	2.90 (0.86)	3.16 (0.85)	-1.48	.141

*p<.05, **p<.01

There were no significant differences between ADHD and control participants in the majority of the demographic variables at baseline. No significant difference was detected between the control participants and the ADHD participants for gender, weight, exercise, fruit and vegetable intake, junk food intake and takeaway intake. Differences were detected at baseline in two demographic variables between the ADHD and the control

[1] 1=Primary school,2=Secondary school,3=TAFE,4=Undergraduate degree, 5=Postgraduate degree. See Appendix II for copy of questionnaire
[2] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week, 5=Daily See Appendix II for copy of questionnaire
[3] 1=Yes, 2=No See Appendix II for copy of questionnaire
[4] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire
[5] 1=Several times a day, 2=Once a day,3=2-3 times a week,4=Once a week,4=Very rarely See Appendix II for copy of questionnaire
[6] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire
[7] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire

participants. These were education level and smoking status. Participants who suffered from ADHD had a significantly lower level of education than control participants and control participants were more likely to smoke than ADHD participants.

Conners

In order to analyse the differences in symptoms of ADHD between control participants and ADHD participants at baseline, an independent groups t-test was conducted. Participant scores for the Conners self-report scale are shown in Table 6-2, and the results of the observers scale can be seen in Table 6-3.

Table 6-2: Conners self-report data by ADHD or Control (Mean and SD).

Conners Subscale	Control	ADHD	t	p
A	12.44 (5.96)	21.36 (8.91)	-5.13	.000**
B	13.29 (7.82)	19.36 (7.82)	-3.54	.001**
C	10.35 (6.21)	18.79 (8.24)	-0.52	.001**
D	5.39 (3.58)	9.71 (5.61)	-4.03	.000**
E	8.63 (4.86)	16.32 (6.90)	-5.71	.000**
F	8.32 (5.35)	15.32 (5.62)	-5.93	.000**
G	16.95 (9.10)	31.65 (10.39)	-7.07	.000**
H	11.09 (6.03)	19.91 (7.03)	-6.32	.000**

*p<.05, **p<.01

Table 6-3: Conners observers report data by ADHD or control (mean and SD).

Conners Subscale	Control	ADHD	t	p
A	11.26 (5.51)	18.04 (7.44)	-3.75	.001**
B	11.56 (5.93)	16.35 (5.03)	-3.19	.002**
C	9.11 (7.06)	18.00 (5.89)	-5.12	.000**
D	5.56 (4.04)	7.92 (4.28)	-2.17	.034**
E	6.73 (4.71)	12.32 (4.29)	-4.75	.000**
F	7.76 (5.12)	13.33 (3.96)	-4.53	.000**
G	14.49 (9.68)	25.54 (8.08)	-4.64	.000**
H	8.00 (4.11)	12.52 (4.07)	-4.28	.000**

*p<.05, **p<.01

Results showed significant differences were detected at baseline in all of the subscales of the Conners scales between groups, with ADHD participants having significantly higher scores on all subscales of the measure, on both the self-report and observer scales. This finding is further strengthened by the result of the inconsistency index, with none of the internal inconsistency measures being significant.

Mood

In order to analyse the differences in mood between control participants and ADHD participants at baseline, an independent groups t-test was conducted. Mood variables for the control participants and the participants with ADHD are shown in Table 6-4 and further shown in Table 6-4.

Table 6-4: Means and standard deviations of mood variables as measured by the Conners scale across Control and ADHD at baseline.

	n (Control/ADHD)	Control	ADHD	t	p
Depression	89 (54/35)	5.07 (5.54)	7.34 (6.66)	-1.74	.085
Anxiety	88 (53/35)	5.04 (5.03)	6.91 (6.07)	-1.58	.118
Stress	89 (54/35)	9.02 (7.45)	15.02 (8.49)	-3.52	.001**

*p<.05, **p<.01

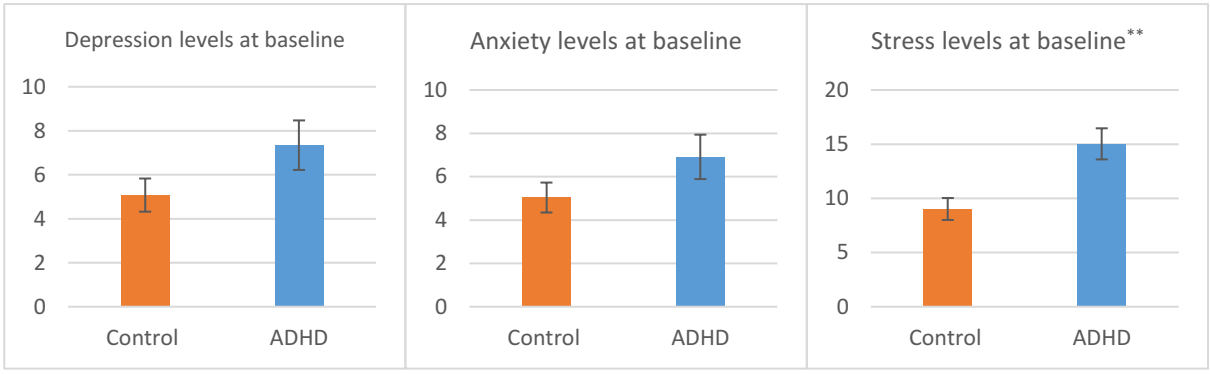


Figure 6-1: Depression, anxiety and stress levels at baseline by control and ADHD, as measured by the Conner's scale.

Independent groups t-tests found that there were no differences in baseline scores between ADHD and control groups for depression or anxiety, however there was a significant difference in stress levels between the groups.

Bloods

In order to analyse the differences in EFA blood levels between control participants and ADHD participants at baseline, an independent samples t-test was conducted. Levels of essential fatty acids, along with the results of the t-test, for the control participants and the participants with ADHD are shown in Table 6-5.

Table 6-5: Means and standard deviations of essential fatty acid measures across control and ADHD at baseline.

	n	Control (n=55)	ADHD (n=35)	t	p
EPA (% of tfa)	90	0.67 (0.26)	0.79 (0.33)	-2.01	.048*
DHA (% of tfa)	90	3.48 (1.11)	3.53 (1.18)	-2.08	.836
ALA (% of tfa)	90	0.21 (0.19)	0.27 (0.19)	-1.20	.234
Omega-3 Index (% of EPA+DHA of total omega-3)	90	4.15 (1.22)	4.32 (1.44)	-6.18	.538

LA (% of tfa)	89	0.03 (0.03)	0.04 (0.05)	-1.52	.133
AA (% of tfa)	90	12.33 (2.34)	11.51 (1.89)	1.81	.073

*p<.05, **p<.01 tfa=total fatty acids

Baseline blood EFA levels were investigated to determine whether there were any differences between control and ADHD participants. Results showed that there were no significant differences between control participants and ADHD participants in levels of DHA, AA, LA, ALA, omega-3 index or EPA/DHA ratio. There was a significant difference in EPA levels at baseline between groups with ADHD participants having higher levels of EPA than controls. This is further demonstrated in Figure 6-2 below.

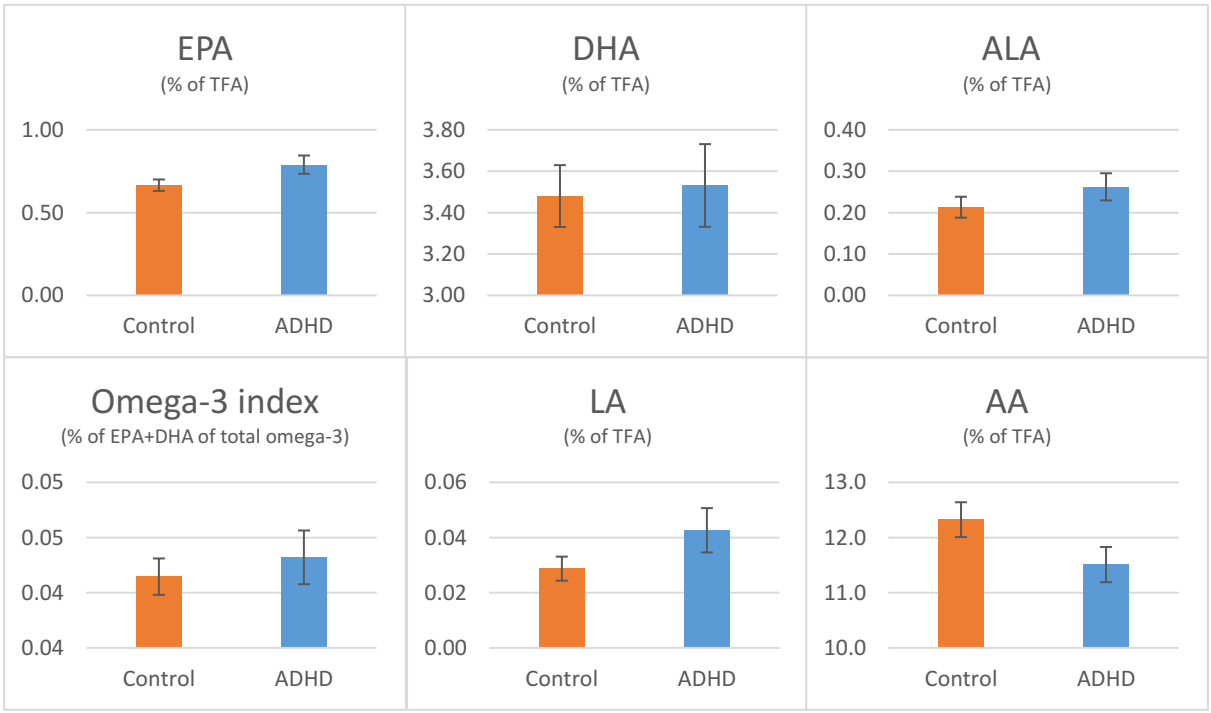


Figure 6-2: Means and standard deviations of essential fatty acid measures across Control and ADHD at baseline.

Cardiovascular Data

In order to analyse the differences in cardiovascular performance at baseline, blood levels between control participants and ADHD participants at baseline, an independent samples t-test was conducted.

Levels of cardiovascular performance, along with the results of the t-test, for the control participants and participants with ADHD are shown below in Table 6-6.

Table 6-6: Baseline cardiovascular data by Control/ADHD.

	Control		ADHD				
	Mean	SE	Mean	SE	t	df	p
Systolic pressure (mmHg)	124.18	1.77	126.52	3.22	-0.64	52	0.53
Diastolic pressure (mmHg)	76.96	1.49	81.58	2.00	-1.86	87	0.07
Pulse pressure (mmHg)	47.21	1.41	44.94	2.09	0.93	87	0.35
Central systolic pressure (mmHg)	109.23	1.69	110.91	2.60	-0.56	87	0.57
Central diastolic pressure (mmHg)	78.13	1.50	83.09	2.06	-1.98	87	0.05
Central pulse pressure (mmHg)	31.11	1.03	27.82	1.25	2.00	87	0.05
Central augmentation index	116.13	2.26	109.97	2.28	1.80	87	0.08
Average common carotid blood flow velocity	22.95	0.61	24.06	0.92	-1.05	84	0.30

*p<.05, **p<.01

From the data above, it can be seen that there were no differences between the control and ADHD participants in the cardiovascular variables measured.

Psychophysics

In order to measure cognitive performance, the participants underwent the SUCCAB cognitive battery and a Stroop task. This battery of tasks was chosen as they provide insight into cognitive processing speed and accuracy of a variety of cognitive domains. This study used the following tasks from the battery, simple reaction time, complex reaction time, immediate and delayed visual memory performance, and spatial working memory performance. Reaction time and accuracy were recorded for each of these tasks. For additional information on these tasks refer to section 5.7.

In order to analyse the differences in cognitive performance between controls and ADHD participants at baseline, a series of independent samples t-tests were conducted. Results are presented in Table 6-7 and Table 6-8.

Table 6-7: Mean accuracy for SUCCAB battery tasks across Control and ADHD at baseline.

Accuracy (%)	n	Control	ADHD	t	p
Simple reaction time	82	96.35 (0.06)	96.91 (0.04)	-0.46	.647
Complex reaction time	91	87.13 (0.11)	85.00 (0.10	0.94	.352
Simple recognition memory	93	73.39 (0.10)	72.88 (0.10)	0.25	.803
Spatial working memory	95	87.28 (0.10)	86.58 (0.08)	0.36	.718
Delayed recognition memory	96	76.21 (0.11)	70.90 (0.11)	2.21	.030*
Congruent Stroop task	37	97.81 (2.50)	94.67 (12.29)	1.12	.271
Incongruent Stroop task	37	94.21 (5.39)	94.85 (5.29)	-0.36	.721
Box Stroop task	37	81.40 (10.61)	70.03 (22.26)	1.93	.067

*p<.05, **p<.01

Table 6-8: Mean reaction time for SUCCAB battery tasks across Control and ADHD at baseline.

RT (ms)	n	Control	ADHD	t	p
Simple reaction time	87	229.42 (42.29)	243.97 (42.70)	-1.56	.123
Complex reaction time	91	368.39 (38.63)	381.02 (45.61)	-1.42	.158
Simple recognition memory	92	920.73 (97.44)	908.14 (79.11)	0.65	.515
Spatial working memory	97	765.70 (128.08)	791.99 (114.04)	-1.03	.306
Delayed recognition memory	96	934.11 (127.25)	935.12 (128.38)	-0.04	.970
Congruent Stroop task	37	742.75 (124.01)	752.41 (114.67)	-2.44	.808
Incongruent Stroop task	37	881.75 (124.31)	802.41 (122.82)	1.95	.060
Box Stroop task	37	1202.85 (138.47)	1071.18 (166.60)	2.63	.013*

*p<.05, **p<.01

Results showed that there were no significant differences between Control participants and ADHD participants in reaction time or accuracy in simple reaction time, complex reaction time, simple recognition memory, spatial working memory or delayed recognition memory. Additionally, no significant difference was detected between the

groups in delayed recognition memory reaction time. A significant difference was detected at baseline in the accuracy of delayed recognition memory between groups, with ADHD participants having an average lower accuracy than control participants. A significant difference was also detected at baseline in the reaction time of the box version of the Stroop task between groups, with ADHD participants having an average lower reaction time than the control participants.

6.7 Discussion

In this chapter, the results of a baseline analysis of blood levels of EFAs, demographic data, mood data, cognition, and cardiovascular variables are presented. Specific hypotheses for each section are presented below.

Demographics

As predicted, the control and ADHD participants were comparable across demographic variables, with no significant differences being detected in the majority of variables. One significant difference that was detected found that control participants had a higher average education level than the ADHD participants. This is perhaps unsurprising given the primary symptoms of the disorder are attentional deficits (American Psychiatric Association, 2013a). This may have contributed to learning difficulties while in school resulting in ADHD participants having a lower education level. While education level may be seen as an extraneous variable, it must be noted that there were no significant differences on the Raven's advanced matrices. The mean Raven's score of all participants was 27, with there being no significant difference between the groups. This supports previous research which found a mean score of 24.65 in a sample of 51 young adults using a timed version of the test (Hamel & Schmittmann, 2006).This suggests that the ADHD participants are comparable in intellectual ability in comparison to the control participants despite having lower education levels.

It was also found that control participants were more likely to smoke than ADHD participants. The p-value for this finding was only marginally significant, with it falling close to the threshold for significance. It is possible this finding was due to multiple comparisons and is not meaningful. However, it is pertinent to check other variables that might be affecting this. As research has shown that males are more likely than females to smoke (Waldron, 1991) and the sample was predominantly male, a t-test was performed to investigate whether there was a difference between the genders in smoking status. As there was no significant difference in smoking status between the genders in either the

ADHD group, the control group or the entire sample, this may be further evidence of the results being marginal.

Conners Scores

The expected difference between the ADHD and control populations in terms of Conners scores was strongly demonstrated. It was predicted that ADHD participants would score higher than the control participants on the Conners scale. This hypothesis was supported, with significant differences detected between the control and ADHD participants on all eight subscales for both the self-report and the observers scale. This is a very important finding, as it helps strengthen the validity of the study. If the participants were in the ADHD group, it is important that they score higher than the controls on the measurement of these symptoms. The most obvious limitation of this study is in regards to clinical participants and refers to the presence of a clinical diagnosis. The researchers of this study endeavoured to collect a diagnostic report from each participant, written by their clinician confirming the diagnosis of ADHD. At the beginning of the study, the researchers attempted to only recruit adults with a clinical diagnosis of ADHD. However, due to logistical problems in some cases, this report was difficult to obtain and therefore the researchers could not confirm the diagnosis of some participants in this study. Researchers asked every ADHD participant for a copy of their diagnostic report, but possibly due to the nature of the condition, not every participant was compliant with this request. This request was followed up by emails and telephone calls, but after numerous attempts the researchers still accepted the participants into the study. The criteria became the presence of ADHD symptoms rather than a formal diagnosis of ADHD. It is also important to ensure the control participants do not have undiagnosed ADHD. The findings of the Conners scale in this study indicate that there was a significant difference in ADHD symptoms between the control and ADHD participants despite not having official diagnoses for all participants.

DASS scores

As previous research has found that ADHD often presents with co-morbid disorders (Gillberg, 1998; Torgersen, Gjervan, & Rasmussen, 2006), it was predicted that ADHD participants would have high levels of depression, anxiety and stress. The hypothesis was not fully supported with scores for both groups on all subscales were within one standard deviation of norms established by previous research (Lovibond & Lovibond, 1995). There are many reasons why the hypothesis may not have been supported. It may have been that the cohort of ADHD participants that were recruited were high functioning. While they did suffer from the symptoms of ADHD, as evidenced by their scores on the Conners

rating scale, their capability to participate in the study indicates their ability to manage their symptoms. This may indicate an ability to manage symptoms of depression and anxiety and could potentially explain why they did not have high scores on the subscales.

It is interesting to note that while the depression and anxiety scores did not significantly between the ADHD and control participants, the results were trending in the expected direction. Participants with ADHD did exhibit higher levels of both depression and anxiety than controls, although this did not reach statistical significance.

Bloods

As predicted, and determined through the omega-3 index score, the levels of essential fatty acids were lower than the ideal levels within the sample population. Research has shown that an omega-3 index of greater than 8% was associated with the greatest levels of protection against cardiovascular disease, whereas an index of 4% or less was associated with the least amount of protection (Harris & Von Schacky, 2004). Results for this study reflected a value of approximately half the ideal, potentially putting the participants at higher risk of myocardial infarction or sudden cardiac death. While this risk is alleviated slightly due to the younger age of the participants, it suggests the intake of essential fatty acids are not adequate in this population. However, based on previous research it was also predicted that the levels of N6 would be much higher than N3 with previous research estimating the ratio to be approximately 16:1 (Simopoulos, 2006). While the ratio was not equal, the levels of inequality were not as high as predicted.

It was predicted that there would be a difference in EFA levels between the participants with ADHD and the control population. This was based on previous research suggesting that EFA levels differ in children with ADHD compared to matched controls (Stevens et al., 1996; Stevens et al., 1995). The result of this study did not replicate these findings. In contrast to the hypothesis, results showed that there were no significant differences between control participants and ADHD participants in the majority of essential fatty acid levels, with only EPA showing a significant difference between the groups. There was a significant difference in EPA levels at baseline between groups, with ADHD participants having marginally higher levels of EPA at baseline than control participants.

There are many possible reasons the results are in contradiction to the hypothesis. There is an abundance of popular scientific literature suggesting that fish oil is beneficial for health, particularly for attention. As attentional issues are a key symptom of ADHD, this may lead to them consuming more fish or taking supplements. Additionally, as explored in chapter 4, the vast majority of research in ADHD has been conducted in children. It

could be that there is a difference exists in the dietary intake between children and adults. Future studies could investigate whether differences exist between controls and ADHD in terms of dietary intake and the effect this has on blood levels. Once dietary patterns have been accounted for, it can be further established whether the reported differences in EFA level are due to differences in dietary intake or differences in the processing and uptake of the acids.

Cardiovascular

Contrary to the hypothesis, there was no difference detected between the ADHD and control participants on any of the cardiovascular variables except central pulse pressure. Central pulse pressure was found to be significantly higher in control participants than in participants with ADHD, but this result must also be interpreted with caution as it was only marginally significant. These hypotheses were based on the theory that ADHD participants have lower levels of EFAs than their control counterparts. As this hypothesis was not supported either, this could possibly help to explain why no cardiovascular differences were detected between the groups.

Psychophysics

Contrary to the hypothesis, results showed that there were no significant differences in the majority of cognitive tasks between the ADHD and control participants at baseline. There are many possibilities as to why this finding may have been contradictory to the hypothesis. One is that the hypothesis was based on differences being present in the blood EFA levels between ADHD and control participants. While this hypothesis was founded in past research, it was not found in this sample population. This may have been why there were few differences detected in cognitive performance between the two groups.

One significant result that was detected at baseline was in the accuracy of delayed recognition memory between groups, with ADHD participants having an average lower accuracy than control participants. This could be because it contains a longer delay than the rest of the tasks, and other tasks are completed in between. As distractibility is a symptom of ADHD, differences in distractibility may have resulted in poorer encoding of the images at the initial presentation leading to difficulties in recalling these images at a later time. This could be the reason why this is the variable which ADHD participants perform differently to control participants.

The other significant result that was detected at baseline was the box condition reaction time of the Stroop task between groups, with ADHD participants having a lower average reaction time than the control participants. This may be explained by ADHD being

characterised by impulsivity. If this were the case, it might be expected that there would also be a difference in accuracy. While this difference was marginally insignificant ($p=0.06$), it indicates a trend in the data.

In conclusion, the current chapter investigated the effects of baseline EFA levels on cognition and cardiovascular performance in a young adult sample with and without ADHD. The results provided strong evidence that differences were present between the two groups in terms of ADHD symptoms as measured by the Conners scale. While it is important to understand the baseline results, the next chapter will investigate whether a 12 week EFA supplementation affects the blood levels of participants.

Chapter 7 Blood and demographic results

The baseline results of chapter 6 were augmented through a clinical trial of the effects of supplementation on essential fatty acid levels in the blood. Participants were randomly allocated to one of three supplementation groups for a 12-week period, providing an opportunity to further assess the effect of essential fatty acid levels. In this chapter, the effects of essential fatty acid supplementation on blood levels of these acids, both in a healthy control sample and in a sample of participants suffering from ADHD are presented.

In order to operate at a peak level, the body requires an adequate supply of essential fatty acids (Lunn & Theobald, 2006). The previous chapter of this thesis suggested that levels of essential fatty acids in the sample population recruited for this study are lower than recommended levels, while EFA levels in the ADHD population were comparable to that of the Control population. Before the potential benefits of supplementation are investigated, it is important to establish whether supplementation leads to changes in the blood levels of EFAs.

As the findings of chapter 6 findings revealed no differences in baseline blood levels of EFAs between control and ADHD participants, the population as a whole (ADHD and control) has been analysed in terms of supplementation group. This gives an indication of the effects of the acids independent of ADHD status. Additionally, by increasing the sample population, this also increases the power of the study and the validity of any findings.

7.1 Aims

The broad aim of this chapter was to investigate the changes in essential fatty status of the sample after a 12-week supplementation period. More specifically, this chapter had two separate aims relevant to different aspects of the research question. Firstly, the chapter aimed to investigate whether differences exist in EFA changes between control and ADHD participants. Previous research has found that both plasma and red blood cell essential fatty acid levels are lower in people with ADHD than in matched controls (Stevens et al., 1995). This is despite there being no differences in dietary intake of energy, protein, vitamin or carbohydrate intake (Stevens et al., 1995) suggesting that the lower levels of the ADHD participants may not be due to a primary deficiency of the acids. Section 6.6 found that there were no significant baseline differences in EFA levels between control and ADHD participants.

Secondly, this chapter aimed to investigate the difference in EFA levels across the three supplementation groups. This was designed as a compliance check in order to observe the essential fatty acid levels change in the supplementation groups differently to that of the control group.

Based on the findings of previous research and the previous chapter of this thesis, it was predicted that the EFA supplementation would change the omega-3 levels in the blood as measured in the whole blood analysis. This would be displayed by the EPA-rich and the DHA-rich supplementation groups improving their omega-3 levels and ratio, and these changes not being present in the placebo group.

7.2 Methodology

Participants

The sample population was the same as the sample used in the previous chapter. It consisted of 98 participants aged between 18 and 40 years of age, consisting of a control group of participants and a group of participants who suffered from ADHD. The control participants (40 males and 20 females) were aged between 18 and 40 years (M=24.8 years, SD=4.94 years). The participants who suffered from ADHD (19 males and 19 females) were aged between 18 and 36 years (M=24.97 years, SD=4.76 years). All participants had normal or corrected to normal vision, no colour blindness and were free from neurological conditions.

Experimental Design

The present study adopted a double blinded, randomised, placebo controlled parallel design. For details on study design and treatment randomisation refer to sections 5.1 and 5.3.

7.3 Results

Baseline

Health and Demographic Information

Differences Between Control/ADHD

In section 6.6, it was shown that demographic and health variables were comparable across the across the control and ADHD participants. There were no significant differences in age, height, weight or BMI across these comparisons. Control participants had a higher average education level than the ADHD participants and control participants were more likely to smoke than ADHD participants. These values are displayed in Chapter 6 in Table 6-1.

Differences Between Supplementation Groups

Health and demographic variables were found to be mostly comparable between the supplementation groups, with the only significant difference between the supplementation groups being found in junk food intake. There was a significant overall difference detected between the junk food intake of the groups using an ANOVA. This is shown below in Table 7-1. Further analysis detected the specific differences were present when contrasting the EPA-rich group with both of the other groups individually, with EPA-rich participants consuming less junk food than either of the other two groups.

Table 7-1: Demographic data by supplementation group.

	Placebo	EPA-rich	DHA-rich	f	p
Number of participants	36	31	31		
Gender (M/F%)	53/47	71/29	58/42	1.19	0.31
Age	24.06 (4.44)	25.29 (5.36)	25.38 (4.79)	.80	0.45
Height (cm)	171.32 (10.96)	175.27 (9.91)	172.61 (10.44)	1.16	0.32
Weight (kg)	71.87 (16.12)	72.45 (10.84)	69.82 (13.15)	.30	0.75
Body Mass Index (BMI)	24.45 (4.90)	23.57 (2.88)	23.31 (3.16)	.73	0.49
Education Level	3.31 (1.13)	3.03 (1.03)	3.33 (1.09)	.73	0.49
Exercise	3.35 (1.23)	3.52 (1.40)	3.87 (0.82)	1.58	0.21
Smoking Status	1.53 (0.66)	1.62 (0.62)	1.40 (0.62)	.90	0.41
Fish Intake	2.60 (0.91)	2.32 (0.94)	2.40 (1.00)	.75	0.47
Fruit and Vegetable Intake	2.02 (0.98)	2.06 (0.89)	1.93 (0.78)	.18	0.84
Junk Food Intake	3.40 (0.60)	3.42 (0.72)	2.90 (0.92)	4.73	0.01*
Takeaway intake	2.91 (0.89)	3.13 (0.76)	2.97 (0.93)	.54	0.58

*p<.05, **p<.01

Bloods

Differences Between Control/ADHD

Earlier in the thesis, section 6.6 showed that EFA levels of ADHD participants and control participants were comparable at baseline, with no differences detected in terms of essential fatty acid status. These results are presented in Table 6-5.

Differences Between Supplementation Groups

Below are tables looking at the differences between the supplementation groups at baseline. It is important to determine whether baseline differences are present, as if there were differences at baseline this needs to be taken into account when looking at differences after the supplementation period. In order to analyse the differences in essential fatty acid levels between groups at baseline, a one-way analysis of variance was conducted. Results are presented below in Table 7-2.

Table 7-2: Means and standard deviations of essential fatty acid measures across supplementation groups at baseline.

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	F	df	p
EPA (% of tfa)	0.67	0.04	0.67	0.04	0.70	0.05	0.11	2	0.90
DHA (% of tfa)	3.79	0.23	3.21	0.16	3.44	0.21	2.08	2	0.13
ALA (% of tfa)	0.17	0.02	0.29	0.04	0.26	0.04	3.36	2	0.04*
Omega-3 Index (% of EPA+DHA of total omega-3)	4.55	0.27	3.88	0.18	4.14	0.23	2.14	2	0.12
LA (% of tfa)	0.04	0.01	0.03	0.01	0.03	0.01	0.27	2	0.76
AA (% of tfa)	12.22	0.38	11.88	0.41	11.88	0.44	0.26	2	0.77

*p<.05, **p<.01, tfa=total fatty acids

The values in Table 7-2 are shown graphically below in Figure 7-1.

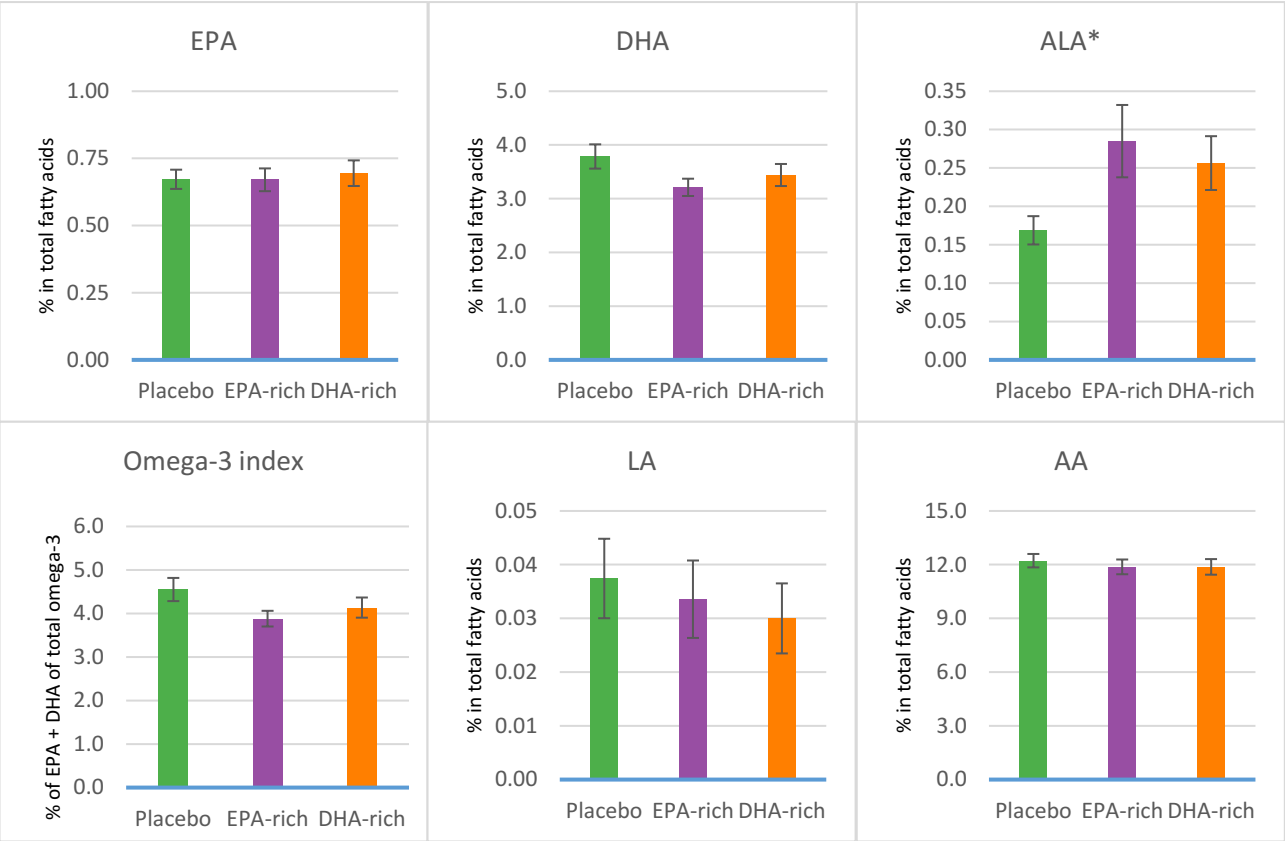


Figure 7-1: Baseline levels of EFAs between supplementation groups (*p<.05, **p<.01).

At baseline, no significant difference was detected between the three supplementation groups for EPA, DHA, AA, LA, omega-3 index or EPA/DHA ratio (P>0.05). This indicated there were no differences in levels of these fatty acids between the three different supplementation groups at baseline. At first analysis, there was a significant relationship between the supplementation groups and ALA levels.

Further contrast tests were conducted, and it was found that significant differences existed between placebo and EPA and placebo and DHA. In order to minimise experiment wise error, the Bonferroni correction was used. As 3 planned comparisons were conducted, this meant that the threshold for rejecting the null hypothesis was 0.016 (0.05 divided by 3). When this is considered, there are no significant differences at baseline in ALA.

After Supplementation

After the baseline levels of cardiovascular were tested, statistical tests were conducted to detect the differences in cardiovascular function after the supplementation period.

Between Groups - ADHD vs. Control

In order to analyse whether control participants and ADHD participants are comparable after supplementation, an independent groups t-test was conducted. Results are presented in Table 7-3 below.

Table 7-3: Means and standard deviations of essential fatty acid measures across control and ADHD participants after supplementation.

	Control		ADHD				
	Mean	SE	Mean	SE	t	df	p
EPA (% of tfa)	1.27	0.12	1.41	0.20	-0.65	83	0.52
DHA (% of tfa)	4.10	0.25	4.41	0.40	-0.70	83	0.49
ALA (% of tfa)	0.33	0.03	0.27	0.02	1.36	83	0.18
HS-omega-3 Index (% of EPA+DHA of total omega-3)	5.36	0.31	5.82	0.51	-0.81	83	0.42
LA (% of tfa)	0.03	0.00	0.03	0.00	0.57	84	0.57
AA (% of tfa)	10.50	0.27	10.55	0.28	-0.13	83	0.90
*p<.05, **p<.01	tfa= total fatty acids						

Similar to baseline, EFA levels of ADHD participants and control participants were also comparable after supplementation, with no significant differences detected in terms of essential fatty acid status.

This is further demonstrated in Figure 7-2 below, which show baseline data compared next to after supplementation data and further display the changes over the supplementation period.

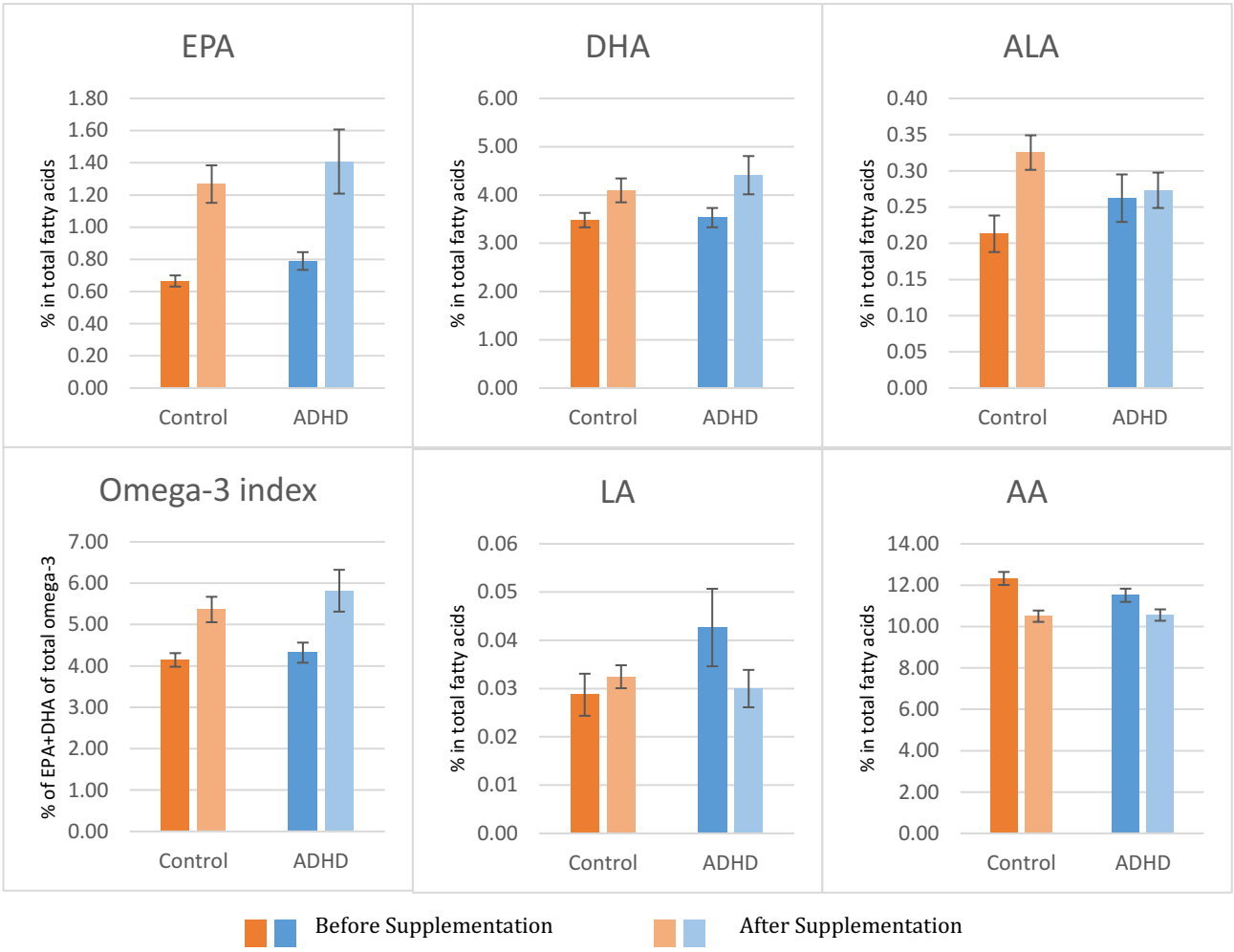


Figure 7-2: Baseline and after supplementation EFA levels by Control and ADHD participants.

The previous chapter showed that there were no baseline differences in EFA levels between control and ADHD participants. Similarly, Table 7-3 above showed that EFA levels after supplementation were similar between ADHD and control participants. The next section looks at the relative difference in EFA levels before and after supplementation. Therefore, further analyses were conducted to determine whether or not there were significant differences between control and ADHD participants in the change in EFA levels as a result of supplementation. An independent samples t-test was conducted and the results are presented below in Table 7-4.

Table 7-4: Means and standard deviations of change in essential fatty acid measures across time points in Control and ADHD participants.

	Control (n=52)		ADHD (n=27)				
	Mean	SE	Mean	SE	t	df	p

EPA (% of tfa)	0.63	0.12	0.63	0.20	0.02	77	0.98
DHA (% of tfa)	0.60	0.28	0.85	0.42	-0.49	77	0.62
ALA (% of tfa)	0.11	0.04	0.00	0.05	1.82	77	0.07
Omega-3 Index (% of EPA+DHA of total omega-3)							
	1.24	0.34	1.48	0.54	-0.39	77	0.70
LA (% of tfa)	0.00	0.01	-0.01	0.01	1.45	37	0.16
AA (% of tfa)	-1.89	0.33	-0.56	0.43	-2.37	77	0.02*

*p<.05, **p<.01 tfa= total fatty acids

Results showed that there were no significant differences after supplementation between the control participants and ADHD participants in the majority of the essential fatty acid changes measured. A significant difference was detected between ADHD and control participants in the difference between baseline and after supplementation AA levels, with levels of AA decreasing significantly more in control participants than in ADHD participants.

Between Groups (Supplementation)

Earlier in this chapter, it was shown that essential fatty acid levels of the three supplementation groups were comparable at baseline, with no significant differences detected. In order to analyse whether the EFA levels in the different supplementation groups were comparable after supplementation, an ANOVA was conducted. Results are presented in Table 7-5 below.

Table 7-5: Means and standard deviations of essential fatty acid measures across supplementation groups after supplementation.

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	f	df	p
EPA (% of tfa)	0.75	0.09	1.91	0.22	1.35	0.11	15.14	2	0.00**
DHA (% of tfa)	3.05	0.16	3.66	0.23	6.16	0.40	36.64	2	0.00**

ALA (% of tfa)	0.33	0.04	0.28	0.02	0.30	0.03	0.70	2	0.50
HS-Omega 3 Index (% of EPA+DHA of total omega-3)	3.79	0.23	5.57	0.39	7.51	0.46	26.48	2	0.00**
LA (% of tfa)	0.03	0.00	0.03	0.00	0.04	0.00	1.27	2	0.29
AA (% of tfa)	10.53	0.38	10.63	0.35	10.38	0.33	0.12	2	0.89

*p<.05, **p<.01 tfa=total fatty acids

As expected, the ANOVA indicated that there were differences between the groups after the supplementation period. This is further demonstrated in Figure 7-3 below, which show baseline data compared next to after supplementation data and further display the changes over the supplementation period.

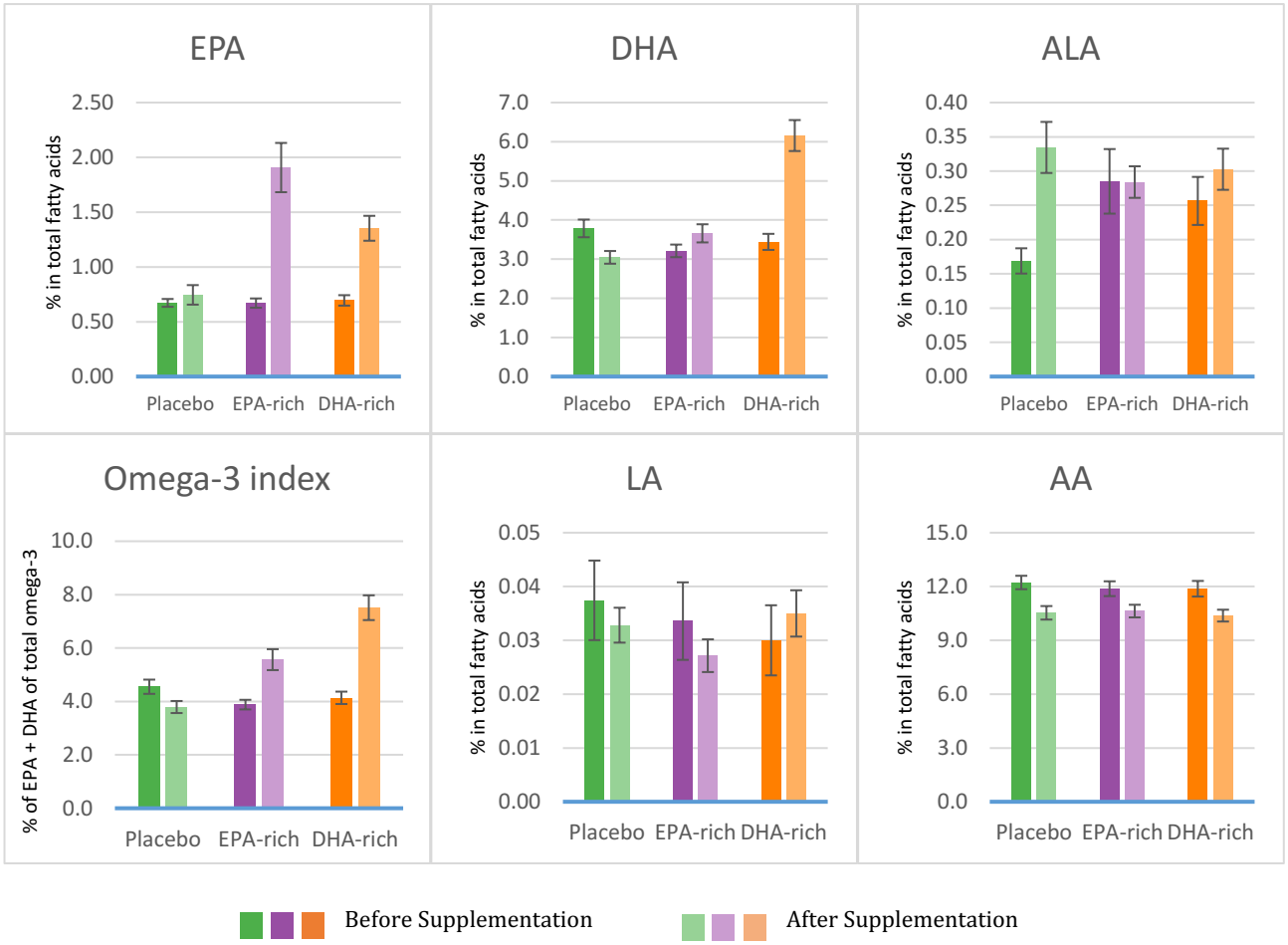


Figure 7-3: Baseline and after supplementation EFA levels by supplementation group.

As can be seen, the levels of EPA, DHA and the omega-3 index did significantly differ across the two time points, indicating the supplementation changed the essential fatty acid levels in the blood. The difference value was calculated and statistical tests were undertaken to see if this value differed between groups. An ANOVA was conducted to determine whether significant differences were present across the two time points between the three groups. Results of this ANOVA are presented in Table 7-6 below.

Table 7-6: Means and standard deviations of changes in essential fatty acid measures across supplementation groups.

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	F	df	p
Difference in EPA (% of tfa)	-0.04	0.07	1.37	0.22	0.71	0.10	26.46	2	0.00**

Difference in DHA (% of tfa)	-0.76	0.18	0.48	0.22	2.71	0.47	34.15	2	0.00**
Difference in ALA (% of tfa)	0.16	0.04	-0.03	0.06	0.08	0.06	3.52	2	0.03*
Difference in HS-Omega-3 Index (% of EPA+DHA of total omega-3)	-0.80	0.19	1.85	0.37	3.42	0.53	35.21	2	0.00**
Difference in LA (% of tfa)	-0.01	0.01	0.00	0.01	0.01	0.01	0.73	2	0.49
Difference in AA (% of tfa)	-1.65	0.39	-1.05	0.49	-1.57	0.56	0.46	2	0.63

*p<.05, **p<.01 tfa=total fatty acids

Consistent with the absolute values collected after supplementation, the levels of change in of EPA, DHA and the omega-3 index did significantly differ across the supplementation groups. Both Table 7-5, Table 7-6 and the figures above indicate that the supplementation led to changes in essential fatty acid levels.

7.4 Discussion

In this chapter, the effect of supplementation on blood levels of essential fatty acids were reported, as well as demographic differences between the groups. Participants were administered with an EPA-rich formulation, a DHA-rich formulation or a placebo formulation for a period of 12 weeks. This chapter explored the effect of EFA supplementation between supplementation groups and between control and ADHD participants.

Demographic Data

The overall goal of recruitment was to obtain a representative demographic sample. As discussed earlier in chapter 6.6, the ADHD and control participants were comparable on most demographic variables. Similarly, the supplementation groups were found to be mostly comparable on demographic and dietary variables, with no significant differences being detected in the majority of variables. One significant difference that was detected found that participants on the EPA-rich supplementation consumed less junk food than

the other two groups. While this could potentially have affected the results, as EPA-rich participants did not have different EFA levels either at baseline or after supplementation, this variable was deemed unlikely to have affected results.

Bloods

The one significant finding between the ADHD and controls in EFA levels across the two time points was a difference in the change of arachidonic acid levels. Despite there being no significant difference in AA between control and ADHD participants at baseline, there was a significant difference after supplementation with AA levels decreasing significantly less in ADHD participants when compared to control participants over the 12-week supplementation period. As discussed in earlier chapter, omega-3s and omega-6s have a competitive relationship, competing for the same position on the phospholipid membrane (Burdge & Calder, 2005). This was observed in the sample population, with omega-6 levels changing as omega-3 levels rose. The results suggest that omega-3 supplementation reduces AA, which is derived from n-6, to a greater extent in controls than in participants with ADHD, perhaps suggesting that the controls are utilising the omega-3s better than the ADHD participants.

This is also supported by previous literature. A meta-analysis found that subjects with learning disorders, including ADHD, had lower levels of red blood cell arachidonic acid (Morse, 2009). This may help to explain why there was a significant change in the difference of AA. If there is an underlying chemical difference in the absorption of AA, then supplementation may make this more apparent despite there being no baseline differences.

This result must be interpreted with caution however, due to the standard error values. The standard error values were much higher in the ADHD sample as compared to the control sample. As standard error is influenced by sample size, it is possible that the difference in the means between the two groups were present due to the control sample being twice the size of the ADHD sample.

While results are interpreted with caution, the difference between the standard error of the groups was the smallest for AA which was the one significant difference detected, which could be interpreted as increasing the validity of the result. The validity of the finding is also increased by the findings of previous studies which suggest red blood cells of AA are lower in samples who suffer from learning disorders (Morse, 2009).

In addition to measuring the differences between ADHD and control participants, differences in blood levels of EFA were also measured between the supplementation

groups. As hypothesised, a 12 week EFA supplementation increased levels of EFAs in the blood, according to the omega-3 index finger prick test. Unsurprisingly, the EPA-rich supplementation led to an increase in EPA whole blood levels, and the DHA-rich supplementation led to an increase in DHA in the blood. Additionally, both supplementation groups had a significant increase in omega-3 index. As EPA, DHA and omega-3 index whole blood levels changed over the supplementation period, this is evidence for compliance to the treatments provided. It also must be noted that both the EPA-rich and the DHA-rich supplementations contained both EPA and DHA. While the ratio was approximately 4:1 for both formulations, it must be noted that due to this, specific mechanisms cannot be isolated entirely to either EPA or DHA.

Also as expected, there were no differences in omega-3 PUFA levels in the placebo group, indicating that the soya bean oil formulation did not affect the omega-3 fatty acid composition of the whole blood cells. This is because the placebo treatment was composed of omega-6 and omega-9 fatty acids. This is also indicated by a capsule count indicating compliance with supplementation was satisfactory. There is limited literature investigating the cognitive effects of soya oil, but the existing literature suggests it is an appropriate placebo treatment to be compared to omega-3 fatty acids. Soya bean oil has also been used as a placebo treatment in numerous other studies investigating the effects of omega-3 fatty acids(DeGiorgio & Miller, 2008; Hamazaki et al., 1996; Tsukada, Kakiuchi, Fukumoto, Nishiyama, & Koga, 2000). Research comparing the effect of DHA supplementation on cerebral blood flow in primates showed the omega-3 supplementation group had greater regional cerebral blood flow than the soya oil controls following tactile stimulation to the right paw. This suggests the DHA plays a greater role between the neuronal activation and the rCBF response to a greater extent than the soya oil (Tsukada et al., 2000).

Interestingly, alongside the increase in omega-3 fatty acids in the blood, there was also a decrease in the omega-6 levels. As discussed in chapter 2, previous research has established that omega-3 and omega-6 fatty acids compete for the same position on the phospholipid membrane (Burdge & Calder, 2005). Omega-3 and omega-6 share the same conversion and elongation enzymes and therefore have a competitive relationship (Lauritzen et al., 2001). This means as the omega-3 levels rise, the omega-6 levels will have less phospholipids to utilise and there will be less omega-6 produced. Hence, if participants are taking omega-3 supplementation, it makes sense that their omega-6 levels would be lower.

There are many different variables that can affect EFA uptake, and it is important to investigate all of these possibilities. It is possible that the dietary intake of participants may also have affected the EFA levels, with previous research finding a high meat diet affected the EPA to DHA conversion to a larger extent than a diet with a high fish intake (Pawlosky et al., 2003). Future research could collect information about the dietary intake of the participants.

7.5 Conclusion

In conclusion, the current chapter showed that 12 weeks of omega-3 supplementation led to an increase in omega-3 EFA levels and a rise in the omega-3 index. In contrast, the placebo supplementation of soya oil did not significantly change levels of EPA or DHA, validating its choice as a placebo.

Chapter 8 Cognitive results

8.1 Abstract

In this chapter, the cognitive effects of the essential fatty acids used in this clinical trial are analysed. By investigating whether the changes in EFA status detected in the blood previous affected cognitive performance, it explores the effect of essential fatty acids on cognition and the difference based upon supplementation group and ADHD status. The present study aimed to evaluate the effect of essential fatty acid supplementation on cognitive performance, both in a healthy control sample and in a sample of participants suffering from ADHD. Results from this chapter reveal that despite relatively few differences in cognitive performance over the supplementation period between control participants and ADHD participants. Given the changes in blood EFA levels detected in the previous chapters, this is perhaps suggestive of a maintenance of performance independent of effects of changes in blood levels.

8.2 Introduction

Previous research has shown a direct link between essential fatty acid status and cognitive function. PUFAs are necessary for the normal growth and development of the brain (Gómez-Pinilla, 2008). It has been suggested that their role in cognitive function (Lunn & Theobald, 2006), may derive from being an essential part in membrane formation for brain cells. Both animal and human studies have found that omega-3 supplementation may result in improved learning and visuospatial memory (Fontani et al., 2005; Su, 2010), although the evidence is not uniform. There is comparatively little research in this area conducted on young adults and particularly those with ADHD, and this study aimed to rectify this lack of research.

As discussed in section 3.2, EFAs have been found to have beneficial effects on cognition, possibly through a combination of anti-inflammatory and cardiovascular mechanisms. They have also been reported to benefit psychiatric disorders such as depression (Sublette, Ellis, Geant, & Mann, 2011) and schizophrenia (Peet, 2003). However, some studies report that despite an increase in functional activation (fMRI) during a sustained visual attention task, this did not translate to improvements in reaction times or accuracy (McNamara et al., 2010). Similarly, DHA supplementation did not provoke significant changes in mental processing and higher order cognitive tasks (Dangour et al., 2010; Jackson, Deary, Reay, Scholey, & Kennedy, 2012; Rogers et al., 2008).

When the effects of baseline EFA levels on cognition were investigated in chapter 6 of this thesis, significant differences between the ADHD and control participants were only

detected in one of the SUCCAB tasks, delayed recognition memory. As chapter 6 of this thesis revealed no differences in baseline cognitive performance between control and ADHD, it was deemed to be appropriate to compare the population as a whole (ADHD and control) in terms of supplementation group. By increasing the sample population, this increases the power of the study allowing an indication of the cognitive effects of the EFAs, independent of ADHD status.

The first results of this chapter will examine the differences between supplementation groups at baseline, determining whether any pre-existing differences were present. Between group differences for control and ADHD participants are presented in chapter 6 of this thesis. After the baseline results are presented, the after supplementation results will be presented for both between groups analyses. This will begin with comparisons between ADHD and control participants then move to comparisons between supplementation groups.

When looking at tasks that measure both speed and accuracy as in the current study, it is important to look at the relationship between the two when considering performance on the task. It has long been established that there is a trade-off between speed and accuracy in many different tasks (Wickelgren, 1977). For example, if speed of response is the criterion, very fast responses are achieved at the expense of accuracy, and similarly best performance is the criterion, then reaction times tend to increase. The issue arises when one examines reaction time versus accuracy in a speeded task. Different tasks have different requirements, and some have clearer concepts of which is more correct than others. For example, in the SUCCAB task simple reaction time, it is clear that the variable of interest is reaction time, as every button press will be correct. However, in the Stroop task, it is a balance of reaction time and accuracy that leads to better performance. This task also highlights individual differences in performance between participants, with more impulsive participants likely to sacrifice correct answers for shorter reaction times. This is useful in a study investigating ADHD, as impulsivity is one of the key symptoms of this disorder (American Psychiatric Association, 2000b).

From this, it can be seen that measuring either reaction time or accuracy in isolation may not always give a complete picture of the participant's cognitive ability, so both should be considered jointly. One possible metric that has been suggested is known as the inverse efficiency score (IES), calculated by dividing the reaction time by the proportion of correct responses (Townsend & Ashby, 1983). While some research has suggested that it may not be appropriate to limit the statistical analysis to just the IES (Bruyer & Brysbaert, 2011), numerous other studies have used it as part of their methodology (e.g.Rach, Diederich, &

Colonius, 2011; Rossignol, Bruyer, Philippot, & Campanella, 2009). After consideration of the pros and cons of this metric, this metric will be explored with caution.

8.3 Methodology

Participants

The sample population was the same as the sample used in the previous chapter. It consisted of 98 participants aged between 18 and 40 years of age, consisting of a control group of participants and a group of participants who suffered from ADHD. The control participants (40 males and 20 females) were aged between 18 and 40 years (M=24.8 years, SD=4.94 years). The participants with ADHD (19 males and 19 females) were aged between 18 and 36 years (M=24.97 years, SD=4.76 years). All participants had normal or corrected to normal vision, no colour blindness and were free from neurological conditions.

Experimental design

The present study adopted a double blinded, randomised, placebo-controlled parallel design. For details on study design and treatment randomisation refer to section 5.3.

8.4 Results

Baseline

Differences Between Supplementation Groups at Baseline

Below are tables looking at the cognitive differences between the supplementation groups at baseline. In order to analyse the differences in cognition between supplementation groups at baseline, a one-way analysis of variance was conducted. Results are presented in Table 8-1 below.

Table 8-1: Mean accuracy for cognitive tasks across supplementation groups at baseline.

	Placebo		EPA-rich		DHA-rich				
	Mean (Acc)	SE	Mean (Acc)	SE	Mean (Acc)	SE	f	df	p
Simple reaction	0.97	0.01	0.97	0.01	0.96	0.01	0.51	2	0.60
Complex reaction	0.87	0.01	0.88	0.02	0.84	0.02	1.18	2	0.31

Simple recognition memory	0.72	0.02	0.73	0.02	0.74	0.02	0.35	2	0.71
Spatial working memory	0.88	0.01	0.87	0.02	0.85	0.02	0.81	2	0.45
Delayed recognition memory	0.74	0.02	0.73	0.02	0.76	0.02	0.39	2	0.68
Congruent Stroop task	0.92	0.1	0.97	1.01	0.99	0.72	1.74	2	0.20
Incongruent Stroop task	0.96	0.0	0.94	5.50	0.94	6.25	.40	2	0.67
Box Stroop task	0.76	0.2	0.72	19.78	81.77	12.05	1.03	2	0.37

*p<.05, **p<.01

Table 8-2: Mean response times for cognitive tasks across supplementation groups at baseline.

	Placebo		EPA-rich		DHA-rich				
	Mean (ms)	SE	Mean (ms)	SE	Mean (ms)	SE	f	df	p
Simple reaction time	240.8	7.35	235.9	9.74	228.0	6.91	0.64	2	0.53
Complex reaction time	382.7	6.98	379.7	7.03	356.9	8.16	3.6	2	0.03*
Simple recognition memory	937.6	17.01	908.9	17.77	898.2	13.27	1.64	2	0.20
Spatial working memory	790.3	20.07	762.7	16.81	772.6	27.65	0.43	2	0.65
Delayed recognition memory	954.7	25.07	918.8	20.64	926.2	19.85	0.75	2	0.48
Congruent Stroop task	769.1	43.84	746.6	31.55	731.6	30.01	0.25	2	0.78
Incongruent Stroop task	846.7	58.14	804.4	28.54	898.8	26.52	1.96	2	0.16

	Placebo		EPA-rich		DHA-rich				
	Mean (ms)	SE	Mean (ms)	SE	Mean (ms)	SE	f	df	p
Box Stroop task	1110.7	52.02	1128.6	47.5	1184.4	38.75	0.61	2	0.56

*p<.05, **p<.01

As hypothesised, no significant baseline differences were detected between the three supplementation groups for the reaction time or accuracy for the SUCCAB tasks of Simple Reaction Time, Simple Recognition Memory, Spatial Working Memory or Delayed Recognition Memory. There were also no differences between the groups in any of the Stroop tasks of congruent, incongruent or box. This indicated there were no differences in these cognitive variables between the three different supplementation groups at baseline. At first analysis, without adjusting for family-wise errors, there was a significant relationship between the supplementation groups and the reaction time for the complex reaction time task. When multiple comparisons were accounted for the threshold for rejecting the null hypothesis was 0.016, and hence there were no significant differences at baseline between the groups for complex reaction time. This can also be seen graphically below in Figure 8-1 and Figure 8-2.

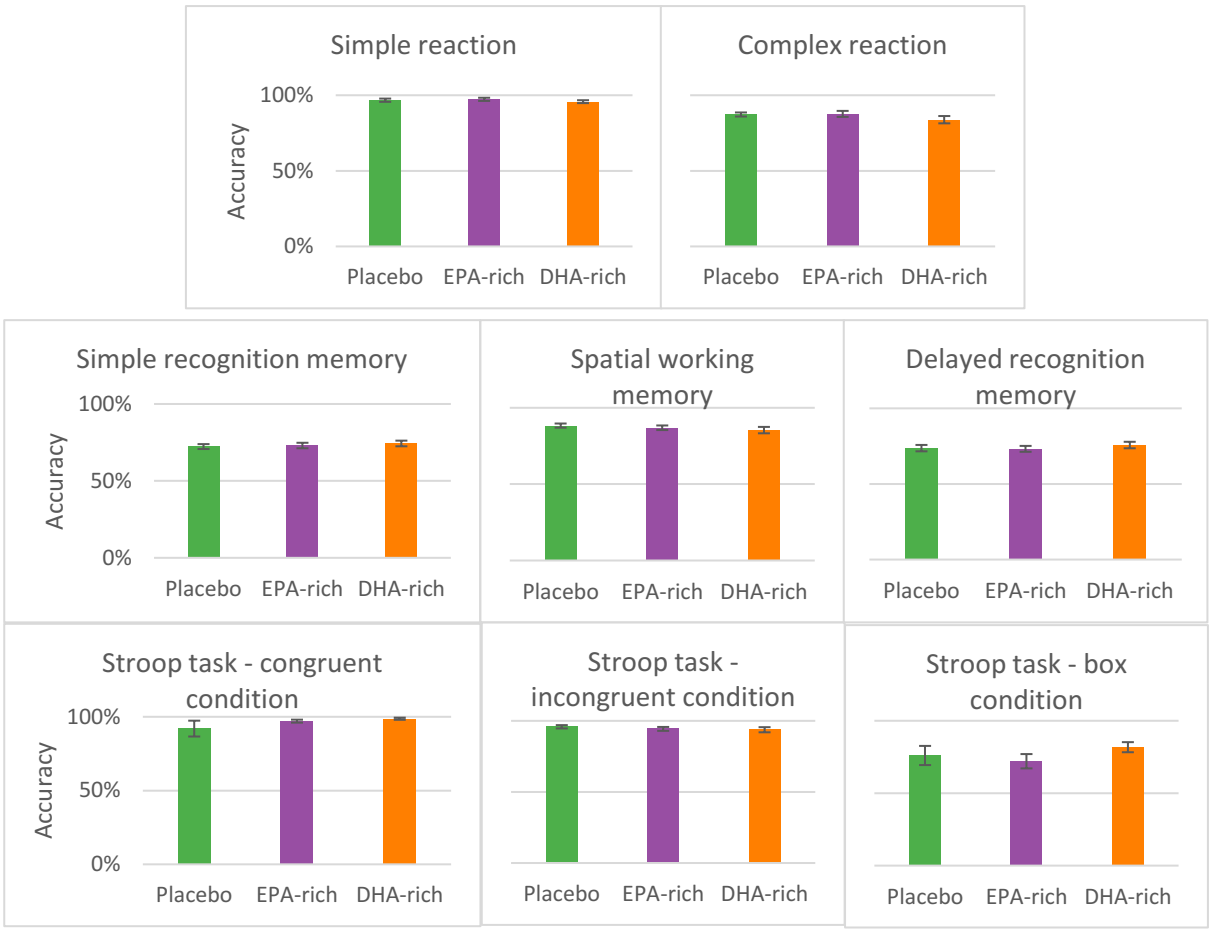


Figure 8-1: Baseline performance on cognitive tasks by supplementation group expressed in percentage accuracy show no differences in accuracy at baseline.

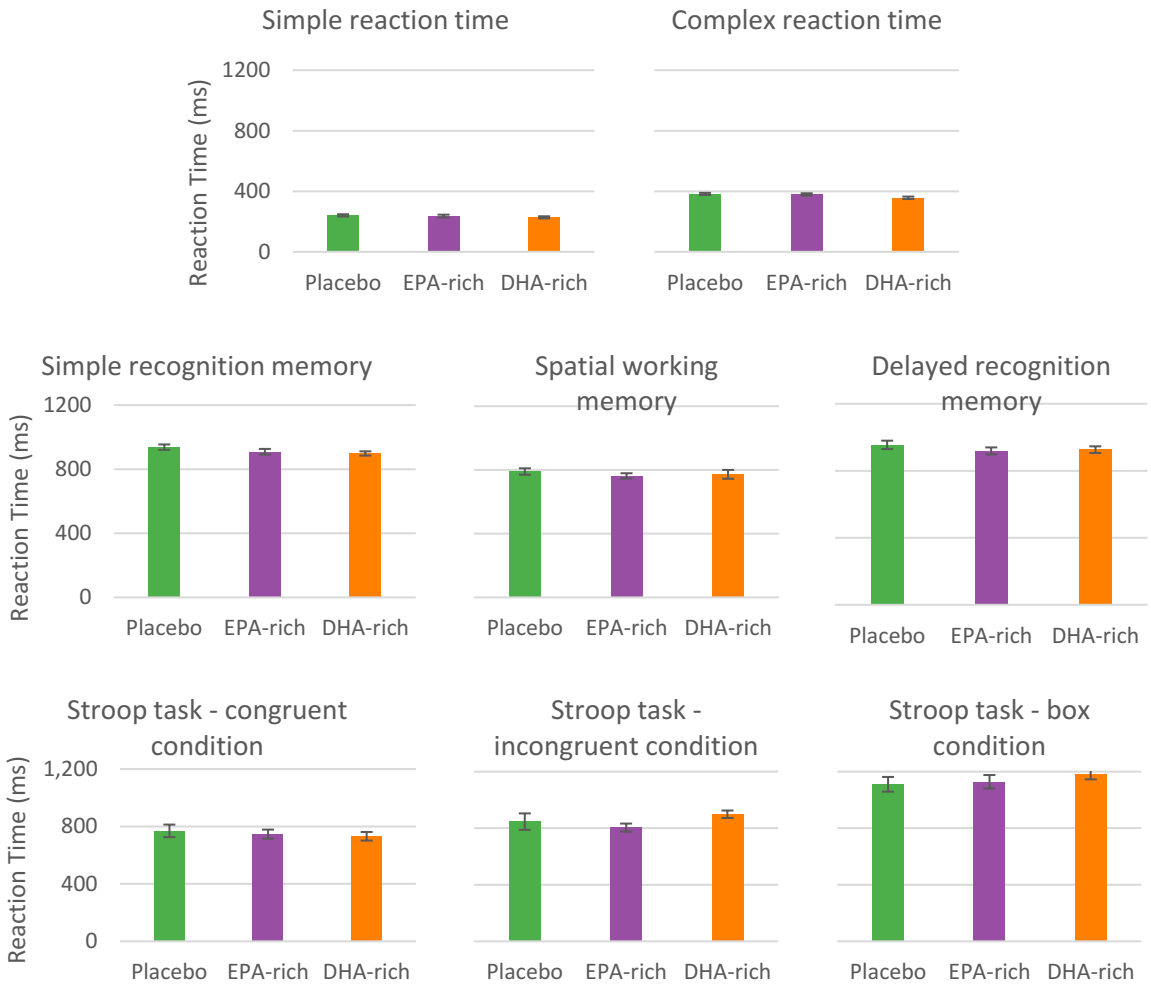


Figure 8-2: Baseline mean reaction times (ms) on cognitive tasks across supplementation group show no differences in reaction time at baseline.

After Supplementation

After the baseline levels of cognitive performance were tested, statistical tests were conducted to detect the differences in cognitive performance after the supplementation period.

Between Groups - ADHD and Control

Participants on placebo supplementation were excluded from this analysis in order to only look at the effect of EFA supplementation between control and ADHD participants, and not have this affected by the presence of placebo participants. In order to analyse cognitive performance after supplementation across the control and ADHD participants, a series of independent groups t-tests were conducted. Results are presented below in Table 8-3 and Table 8-4.

Table 8-3: Mean accuracy for SUCCAB battery tasks across ADHD and Control participants after supplementation.

	Control		ADHD				
	Mean (Acc)	SE	Mean (Acc)	SE	t	df	p
Simple reaction time	0.99	0.01	0.98	0.01	0.50	48	0.62
Complex reaction time	0.88	0.02	0.85	0.03	0.87	52	0.39
Simple recognition memory	0.83	0.01	0.81	0.02	0.84	57	0.41
Spatial working memory	0.90	0.01	0.90	0.02	0.15	57	0.88
Delayed recognition memory	0.79	0.02	0.75	0.02	1.40	56	0.17
Stroop congruent	0.98	0.50	0.95	1.14	2.02	34	.052
Stroop incongruent	0.94	1.48	0.95	1.18	-.54	34	.595
Stroop box	0.87	2.34	0.83	4.28	.96	34	.347

*p<.05, **p<.01

Table 8-4: Mean reaction time for SUCCAB battery tasks across ADHD and Control participants after supplementation.

	Control		ADHD				
	Mean (ms)	SE	Mean (ms)	SE	t	df	p
Simple reaction time	243.1	6.1	266.12	8.3	-2.22	52	0.03*
Complex reaction time	379.4	8.3	395.8	8.6	-1.25	54	0.22
Simple recognition memory	859.3	17.3	874.7	22.4	-0.52	57	0.60
Spatial working memory	725.5	20.0	750.2	22.8	-0.75	57	0.46
Delayed recognition memory	911.9	17.1	896.7	25.2	0.50	56	0.62
Stroop congruent	655.5	22.3	694.4	31.4	-1.04	34	0.31

Stroop incongruent	913.7	30.8	811.3	31.5	2.30	34	0.03*
Stroop box	1199.2	30.6	1094.3	45.7	1.97	34	0.06

*p<.05, **p<.01

This is displayed graphically in Figure 8-3 and Figure 8-4.

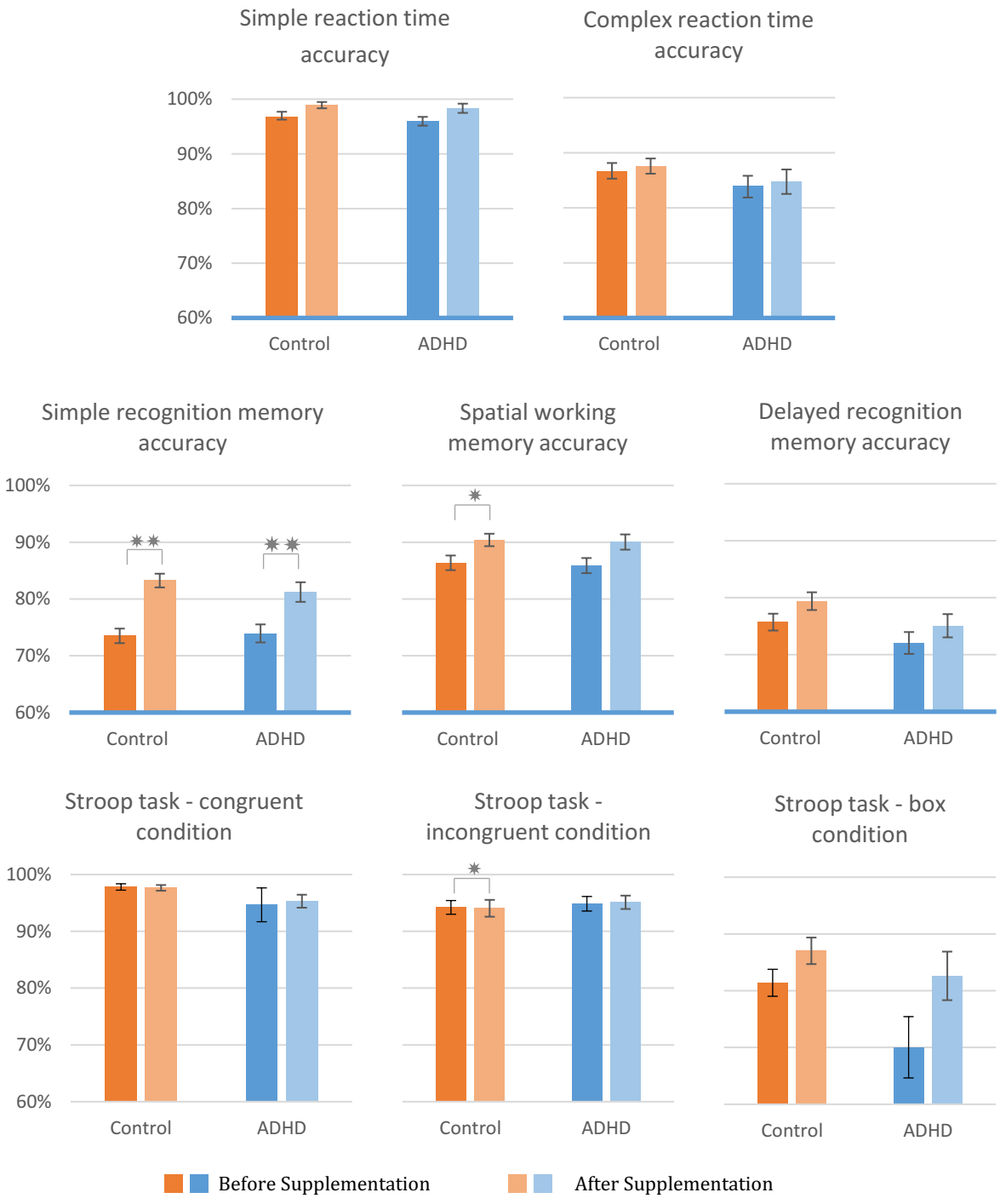


Figure 8-3: Baseline and after supplementation performance on SUCCAB tasks by control and ADHD expressed in percentage accuracy (*p<.05, **p<.01).

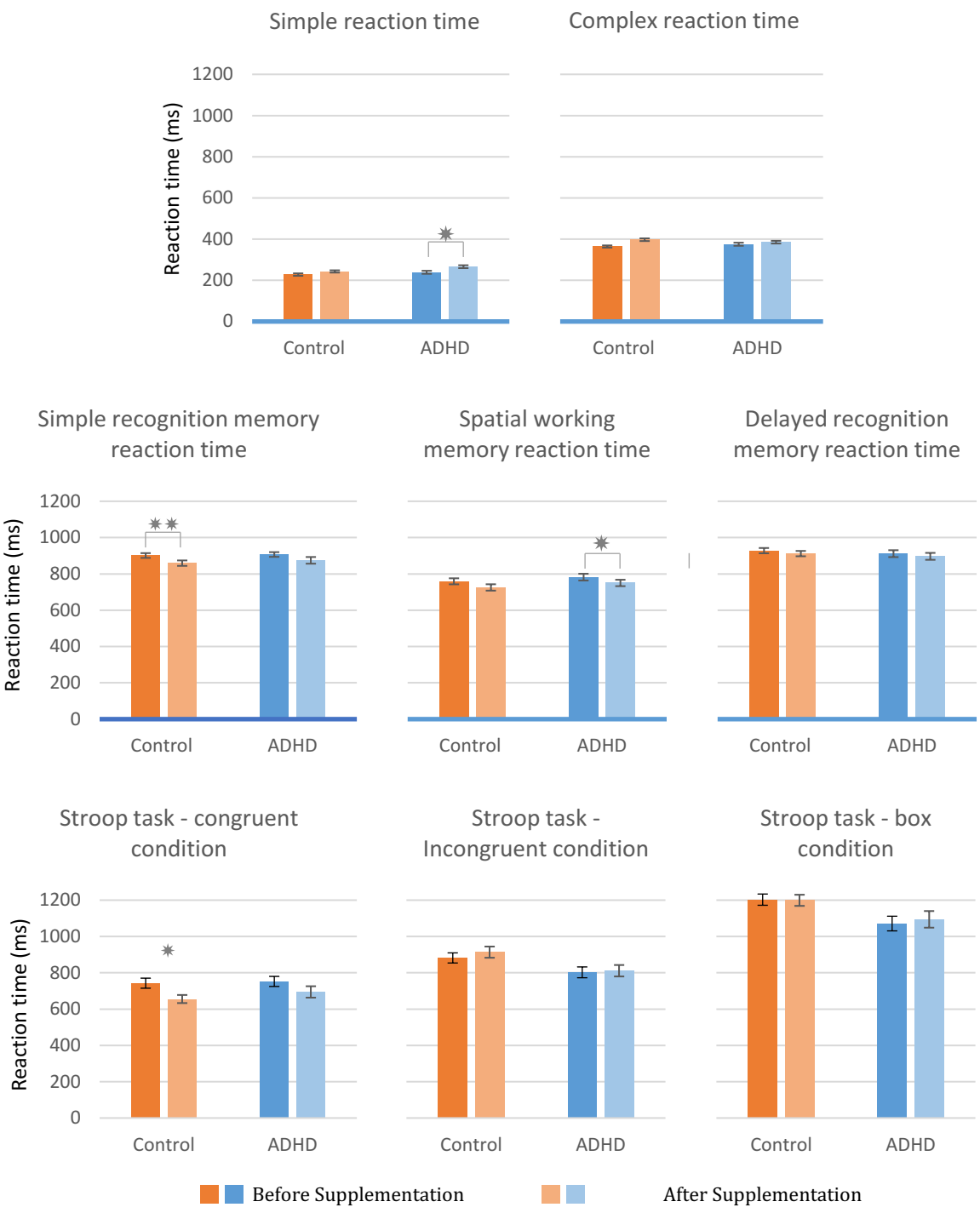


Figure 8-4: Baseline and after supplementation performance on SUCCAB tasks by control and ADHD expressed in reaction time in ms (*p<.05, **p<.01).

Overall, there were no differences in cognitive variables between control and ADHD participants, as measured by the absolute values after supplementation. However, there were some relative differences between the time points for individual variables. As indicated on the graphs above, it can be seen that for the control participants there was a significant difference between the time points for simple recognition memory accuracy

and reaction time, spatial working memory accuracy, and complex reaction time reaction time. For ADHD participants, there was a significant difference between the time points for simple recognition memory accuracy, simple reaction time reaction time and spatial working memory reaction time. This is also summarised in Table 8-5 below.

Table 8-5: Summary of baseline and after supplementation relative cognitive differences between control and ADHD participants.

	Accuracy		Reaction Time	
	Control	ADHD	Control	ADHD
Simple reaction time	NS	NS	NS	✓
Complex reaction time	NS	NS	✓	NS
Simple recognition memory	✓✓	✓✓	✓✓	NS
Spatial working memory	✓	NS	NS	✓
Delayed recognition memory	NS	NS	NS	NS
Stroop congruent	NS	NS	✓✓	NS
Stroop incongruent	✓	NS	NS	NS
Stroop Box	NS	NS	NS	NS

✓: $p < 0.05$, ✓✓: $p < 0.01$, NS: $p >= 0.05$

Reaction Time Divided by Accuracy

After this analysis, another type of post-hoc analysis was undertaken. As discussed above, previous research has found that accuracy divided by reaction time might be another metric that may have validity (Townsend & Ashby, 1983).

Table 8-6: Mean difference in reaction time divided by accuracy for SUCCAB Battery tasks across ADHD and control participants.

	Control		ADHD				
	Mean	SE	Mean	SE	t	df	p
Simple reaction time (baseline)	241.2	7.2	248.5	8.4	-0.93	78	0.35

Complex reaction time (baseline)	427.6	13.7	455.3	19.6	-1.48	88	0.14
Simple recognition memory (baseline)	1250.1	35.4	1243.8	35.9	0.40	90	0.69
Spatial working memory (baseline)	916.0	43.7	903.8	32.0	-0.23	93	0.82
Delayed recognition memory (baseline)	1258.5	44.4	1292.1	51.5	-1.61	94	0.11
Stroop congruent (baseline)	758.7	27.3	817.8	53.6	-1.028	35	0.31
Stroop incongruent (baseline)	940.7	35.7	849.4	35.4	1.803	35	0.08
Stroop box (baseline)	1499.2	54.5	1752.7	207.4	-1.269	35	0.25
Simple reaction time (after suppl.)	250.1	6.1	271.7	9.7	-1.95	76	0.06
Complex reaction time (after suppl.)	441.4	15.2	481.2	26.9	-1.41	80	0.16
Simple recognition memory (after suppl.)	1042.5	26.1	1091.4	42.8	-1.07	86	0.29
Spatial working memory (after suppl.)	817.9	31.5	843.4	37.0	0.57	88	0.57
Delayed recognition memory (after suppl.)	1180.1	42.7	1230.9	70.5	-0.42	86	0.68
Stroop congruent (after suppl.)	671.8	23.6	731.5	36.3	.131	34	0.16
Stroop incongruent (after suppl.)	978.8	41.4	856.7	38.7	.925	34	0.06
Stroop box (after suppl.)	1405.1	63.4	1383.9	91.3	.413	34	0.85

*p<.05, **p<.01

This is displayed graphically below.

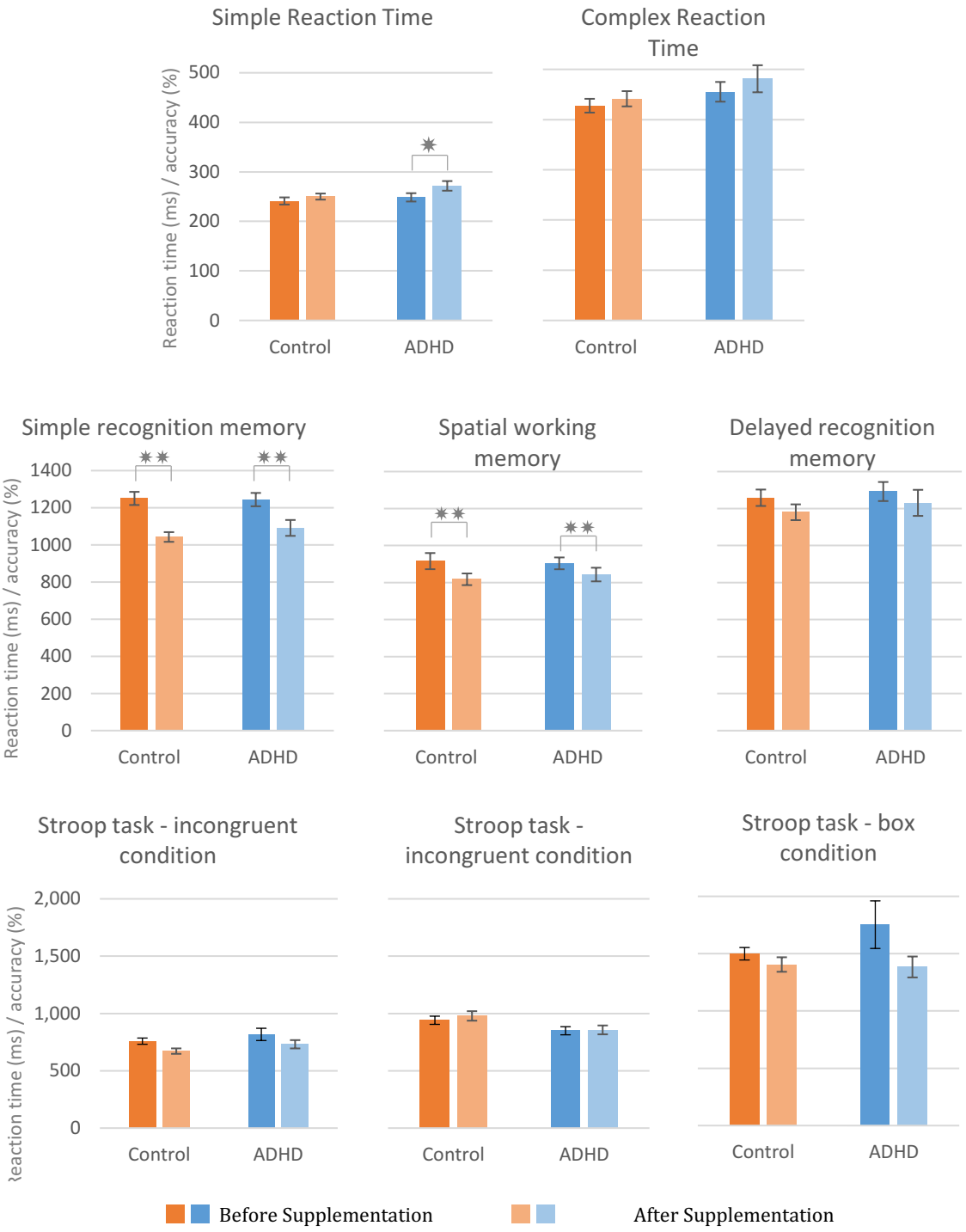


Figure 8-5: Baseline and after supplementation reaction time (ms) divided by accuracy (%) on SUCCAB tasks by control and ADHD (*p<.05, **p<.01).

As indicated on the graphs above, when examining reaction time divided by accuracy, significant differences were detected between the time points for simple recognition memory and spatial working memory for control participants. For ADHD participants, there was a significant difference between the time points for reaction time divided by

accuracy for simple reaction time, simple recognition memory and spatial working memory.

Between Groups (Supplementation)

In order to analyse cognitive performance after supplementation across the supplementation groups, a series of one-way ANOVAs were conducted. Results are presented below in Table 8-7.

Table 8-7: Mean accuracy for SUCCAB Battery tasks across supplementation groups after supplementation.

	Placebo		EPA-rich		DHA-rich				
	Mean (Acc)	SE	Mean (Acc)	SE	Mean (Acc)	SE	f	df	p
Simple reaction time	0.97	0.01	0.98	0.01	0.99	0.0	2.30	2	0.11
Complex reaction time	0.88	0.02	0.87	0.02	0.86	0.0	0.15	2	0.86
Simple recognition memory	0.82	0.02	0.81	0.02	0.84	0.0	0.79	2	0.46
Spatial working memory	0.89	0.01	0.89	0.02	0.92	0.0	1.33	2	0.27
Delayed recognition memory	0.74	0.02	0.78	0.02	0.78	0.0	0.96	2	0.39
Stroop congruent	0.96	0.01	0.97	0.01	0.97	0.01	0.62	2	0.55
Stroop incongruent	0.94	0.02	0.94	0.01	0.95	0.01	0.04	2	0.97
Stroop box	0.84	0.04	0.87	0.04	0.86	0.03	0.13	2	0.88

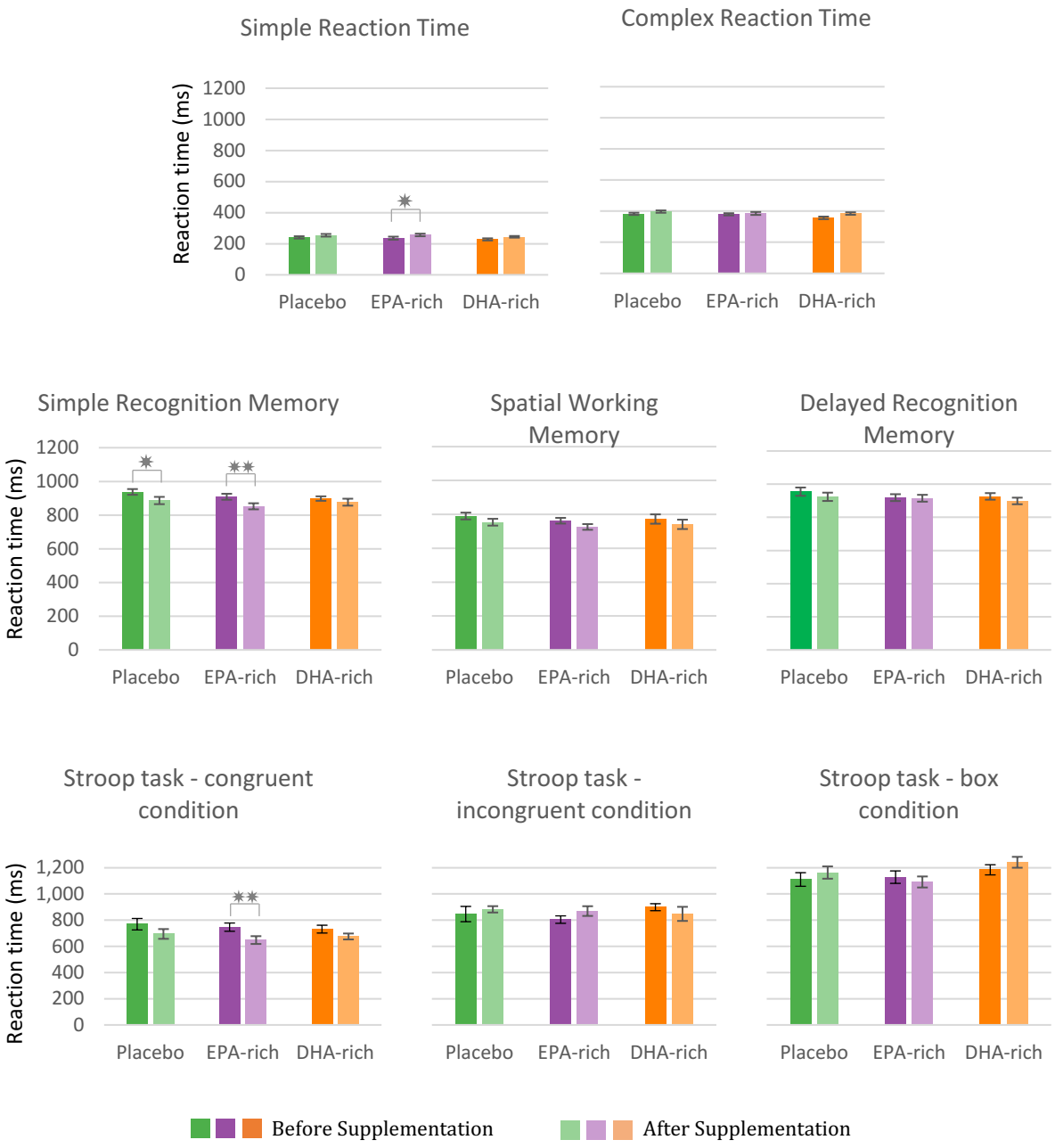
*p<.05, **p<.01

Table 8-8: Mean response times for SUCCAB Battery tasks across supplementation groups after supplementation.

	Placebo		EPA-rich		DHA-rich				
	Mean (ms)	SE	Mean (ms)	SE	Mean (ms)	SE	f	df	p
Simple reaction time	254.5	8.43	256.5	8.32	244.6	5.4	0.68	2	0.51
Complex reaction time	397.2	7.55	385.2	9.54	384.7	8.0	0.71	2	0.49
Simple recognition memory	886.5	21.59	852.3	18.07	876.7	20.7	0.77	2	0.47
Spatial working memory	753.9	24.26	725.9	21.50	741.3	22.3	0.39	2	0.68
Delayed recognition memory	923.8	21.03	915.2	20.01	898.7	20.0	0.39	2	0.68
Stroop congruent	694.8	37.27	648.0	29.85	675.4	22.7	0.61	2	0.55
Stroop incongruent	882.7	24.21	869.4	37.11	848.0	53.8	0.18	2	0.84
Stroop box	1163.4	46.79	1091.7	42.06	1242.0	41.6	2.96	2	0.07

*p<.05, **p<.01

This is presented graphically in Figure 8-6, Figure 8-7



and Table 8-9.

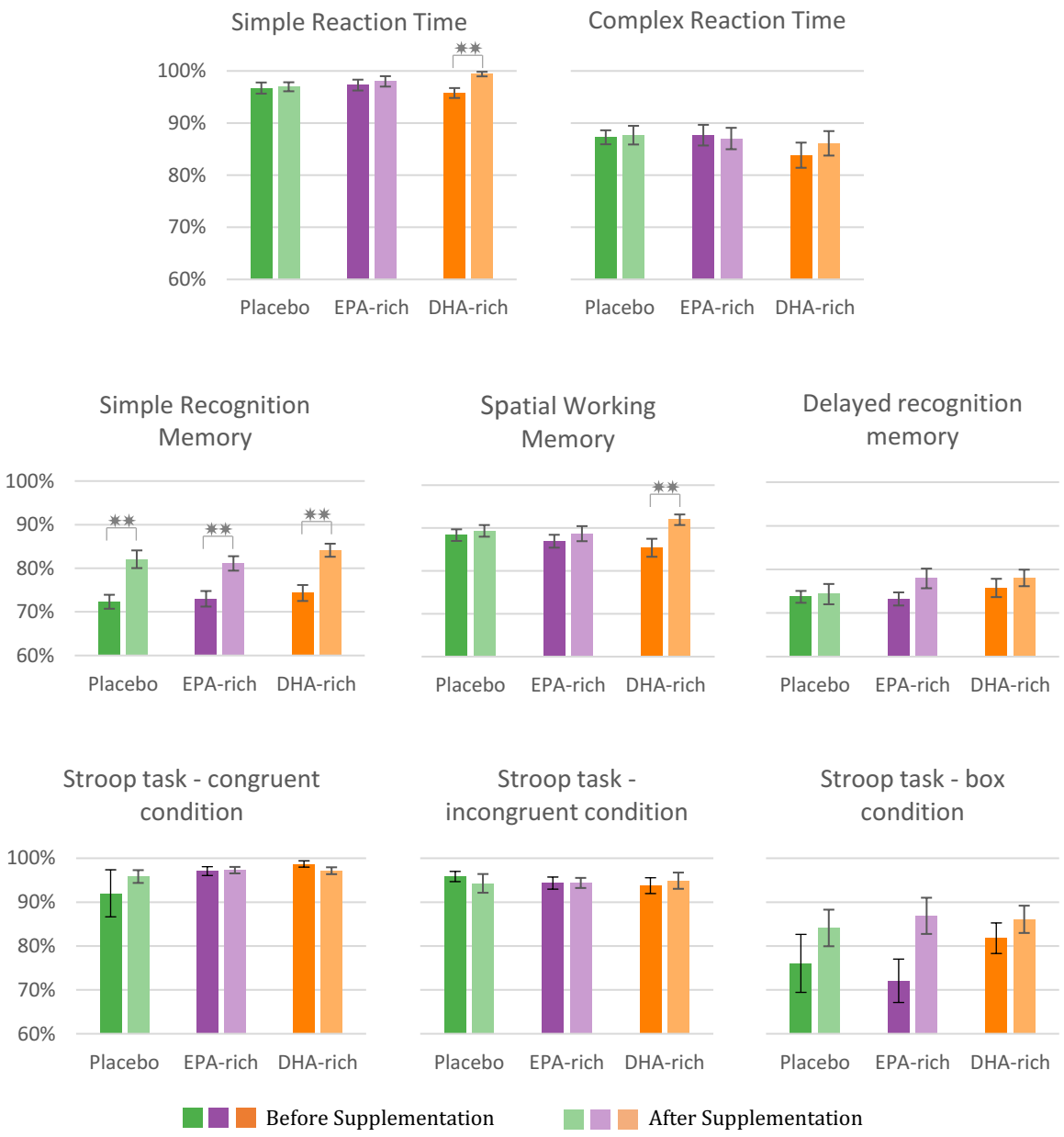


Figure 8-6: Baseline and after supplementation performance on SUCCAB tasks by supplementation group expressed in percentage accuracy (*p<.05, **p<.01).

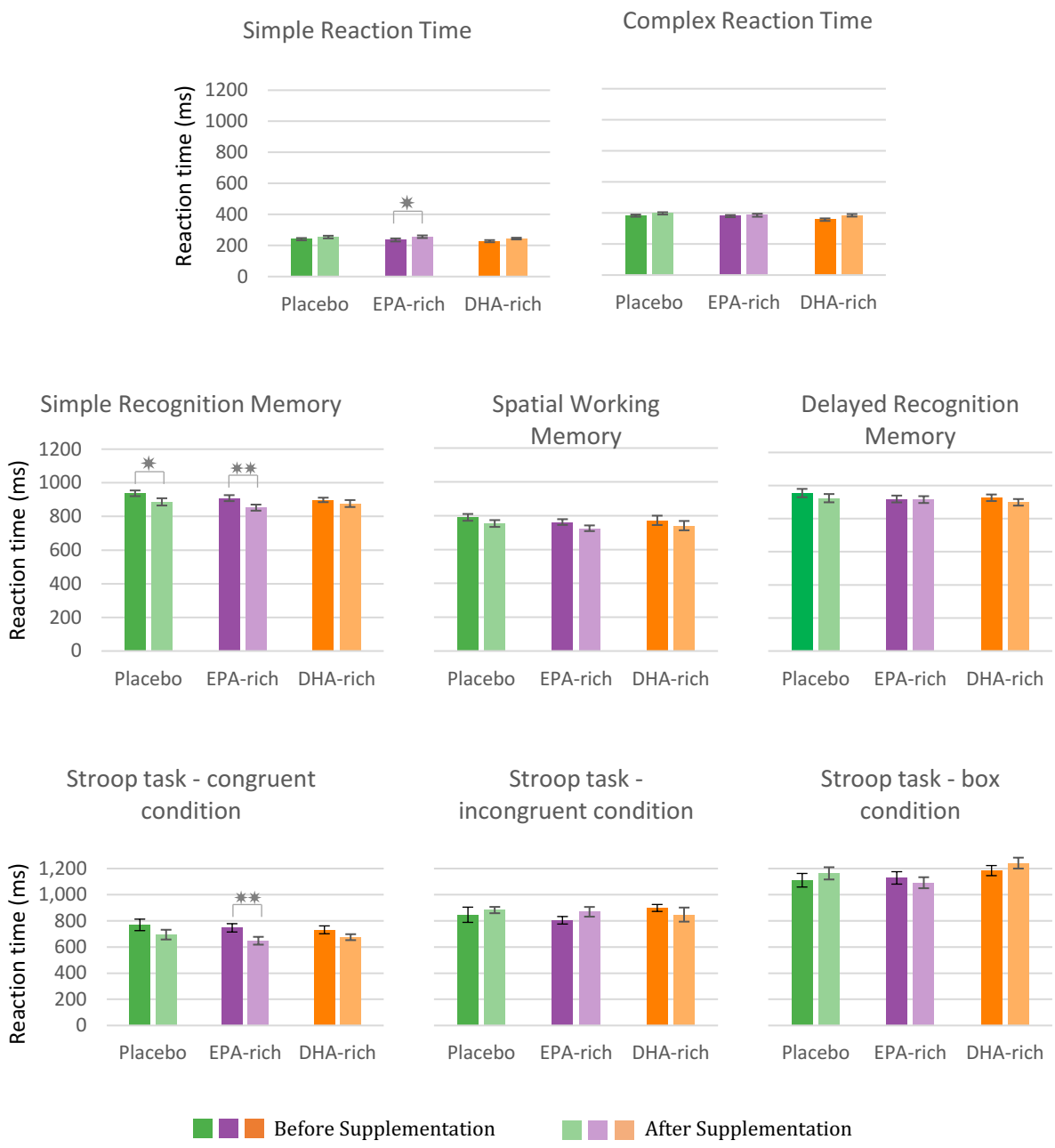


Figure 8-7: Baseline and after supplementation performance on SUCCAB tasks by supplementation group expressed in reaction time in ms (*p<.05, **p<.01).

Table 8-9: Summary of baseline and after supplementation cognitive differences for reaction time divided by accuracy.

	Accuracy			Reaction time		
	Placebo	EPA-rich	DHA-rich	Placebo	EPA-rich	DHA-rich
Simple reaction time	NS	NS	✓✓	NS	✓	NS
Complex reaction time	NS	NS	NS	NS	NS	NS
Simple recognition memory	✓✓	✓✓	✓✓	✓	✓✓	NS
Spatial working memory	NS	NS	✓✓	NS	NS	NS
Delayed recognition memory	NS	NS	NS	NS	NS	NS
Stroop-congruent	NS	NS	NS	NS	✓✓	NS
Stroop-incongruent	NS	NS	NS	NS	NS	NS
Stroop-box	NS	NS	NS	NS	NS	NS

✓: $p < 0.05$, ✓✓: $p < 0.01$, NS: $p >= 0.05$

As indicated on the graphs above, for the participants on placebo supplementation, there was a significant difference between time 1 and time 2 for the Simple Recognition Memory accuracy and reaction time parameters.

For participants on EPA-rich supplementation, there was a significant difference between the time points for simple recognition memory accuracy and reaction time and simple reaction time.

For participants on DHA-rich supplementation, there was a significant difference between the time points for simple reaction time accuracy, simple recognition memory accuracy and spatial working memory accuracy. No significant differences in reaction time were detected for any of the cognitive variables between the time points for the DHA-rich participants.

Reaction Time Divided by Accuracy

As was done above with the ADHD and control groups, another type of analysis was undertaken. As discussed above, previous research has found that accuracy divided by

reaction time might be another metric that may have validity (Townsend & Ashby, 1983). Results are shown in Table 8-10 and Table 8-11 below.

Table 8-10: Mean difference in reaction time divided by accuracy for cognitive tasks across supplementation groups (Baseline).

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	f	df	p
Simple reaction time	245.7	5.0	251.1	8.9	237.7	6.4	1.0	2	0.39
Complex reaction time	442.7	11.9	442.8	16.7	433.9	15.7	0.1	2	0.89
Simple recognition memory	1316.1	36.4	1265.9	37.9	1230.2	35.2	1.4	2	0.24
Spatial working memory	906.2	30.4	891.4	32.1	934.1	53.1	0.3	2	0.74
Stroop congruent	872.8	91.17	771.2	35.39	740.0	27.46	1.7	2	0.21
Stroop incongruent	886.5	66.72	855.7	33.47	965.5	41.42	1.8	2	0.19
Stroop box	1573.5	179.5	1731.0	195.2	1493.7	108.6	.53	2	0.59
Delayed recognition memory	1334.4	54.1	1284.5	46.4	1255.0	50.0	0.64	2	0.53

*p<.05, **p<.01

Table 8-11: Mean difference in reaction time divided by accuracy for cognitive tasks across supplementation groups (After supplementation).

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	f	df	p
Simple reaction time	265.9	7.7	270.3	8.7	245.4	5.5	3.1	2	0.05
Complex reaction time	460.1	17.4	452.8	19.7	458.0	19.5	0.04	2	0.96
Simple recognition memory	1095.0	39.0	1067.5	35.6	1048.6	27.5	0.45	2	0.64
Spatial working memory	854.3	34.6	836.6	36.6	815.3	32.4	0.32	2	0.73

Delayed recognition memory	1277.5	50.5	1215.8	57.0	1177.1	46.6	0.96	2	0.39
Stroop congruent	730.4	47.0	666.5	30.83	694.7	21.2	.88	2	0.42
Stroop incongruent	943.7	39.0	924.5	43.40	901.9	70.24	.15	2	0.86
Stroop box	1419.0	88.1	1311.0	91.59	1463.2	75.50	.83	2	0.44

*p<.05, **p<.01

This is displayed graphically in Figure 8-8 and Table 8-12.

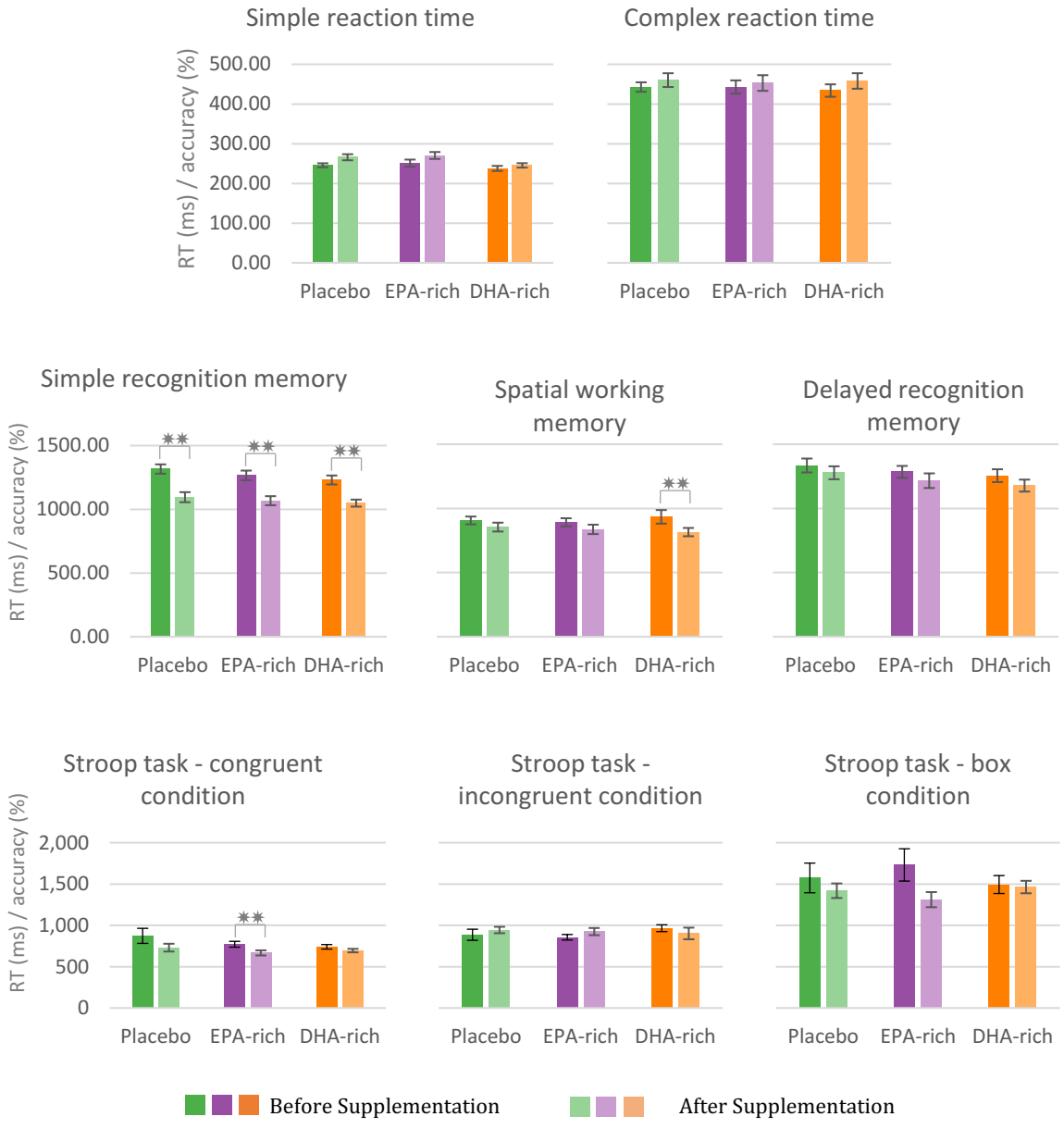


Figure 8-8: Baseline and after supplementation reaction time (RT) in ms divided by accuracy (%) on SUCCAB tasks by supplementation group (*p<.05, *p<.01).

Table 8-12: Summary of baseline and after supplementation cognitive differences for reaction time divided by accuracy.

	Reaction time divided by accuracy		
	Placebo	EPA-rich	DHA-rich
Simple reaction time	NS	NS	NS
Complex reaction time	NS	NS	NS
Simple recognition memory	✓✓	✓✓	✓✓
Spatial working memory	NS	NS	✓✓
Delayed recognition memory	NS	NS	NS
Stroop congruent	NS	✓✓	NS
Stroop incongruent	NS	NS	NS
Stroop box	NS	NS	NS

✓: $p < 0.05$, ✓✓: $p < 0.01$, NS: $p >= 0.05$

When looking at cognitive performance in terms of reaction time divided by accuracy, all three supplementation groups showed a difference between the time points for simple recognition memory. DHA-rich participants also showed a significant difference between the time points for simple reaction time and spatial working memory.

8.5 Discussion

The current study explored the cognitive effects of a 12-week EFA supplementation on both control participants and participants who suffered from ADHD. Based on previous research (Fontani et al., 2005; Kidd, 2007), it was predicted that EFA supplementation would improve cognitive performance in terms of increased accuracy and decreased reaction times. Further, it was predicted that EPA-rich supplementation would improve cognitive performance to a greater extent than DHA-rich supplementation.

In order to further investigate if ADHD status is related to the effect of EFA supplementation on cognition, the placebo participants were removed. Once the placebo participants were removed from the sample, the analysis was run. Overall, there were no differences between control and ADHD participants after supplementation. Some differences were detected between the control and ADHD participants between the two time points. Simple recognition memory reaction time decreased across the time points

for the control participants but not the ADHD population. This could indicate that controls are metabolising the supplementation differently to the ADHD participants and this is leading to the differences in cognitive performance. This difference in metabolism would be in line with previous research suggesting that EFA levels are lower in ADHDs than controls, despite the ADHD sample consuming more EFAs than controls (Stevens et al., 1996; Stevens et al., 1995). In order to further investigate this reaction time was divided by accuracy, creating a new performance variable. This variable was significant for both control and ADHD participants. This suggests that the relationship between reaction time and accuracy is different for the ADHD and control participants but suggests that the EFA supplementation is helping both groups improve even if this is in a different way.

When looking at the results from a supplementation group perspective, simple recognition memory was the cognitive variable that showed the most differences pre and post supplementation. A significant difference was detected in all three supplementation groups for accuracy of this variable and the placebo and EPA-rich supplementation groups showing significant differences in reaction time. While this difference was significant, it may be due to a practice effect. Consistent with this view was the improvement in accuracy of the controls as well as the two supplementation groups, and the reduced reaction times for the controls and EPA-rich participants. If a practice effect did occur, there are two types of practice effect that could have occurred, familiarity with the stimuli and familiarity with the task. Due to an alternate set of stimuli being used for the second testing session, and the order being counterbalanced between participants, it is likely that familiarity with the task may have affected results. The first time the participants did the task, it was novel and they did not know what to expect. The second testing came with a familiarity with the task, and it appears that this may have created a practice effect.

This theory that the improvement if results is due to a practice effect is also supported by the finding that the reaction time divided by accuracy score significantly improved in all three groups for this variable. If these results were due to the EFA supplementation, it would be expected that the controls performance would have stayed the same, or the supplementation groups would have improved to a greater extent than the controls. It is interesting to note that this was only observed in the simple recognition memory task, and not the spatial memory or delayed memory tasks. This perhaps displays the difference of cognitive functions employed by the tasks and the susceptibility of each to possible practice effects.

It is also possible that these results were not due to practice effects at all. There was a 12-week time period between testing sessions, and this is a long time for the participants to recall these abstract images for. Also, a different set of images was used for each session, making it difficult for practice effects to occur. If a practice effect existed for this task, then it could be argued that this effect would be present in all tasks where a speeded response was required, which is all tasks with a reaction time component.

The accuracy of simple reaction time improved in the DHA-rich group, but not in the other two groups. While on the surface that looks like an interesting result, as the accuracy levels were approaching ceiling values in the first testing session, one must consider if a minimal improvement is important, even if it is significant.

One explanation for the findings of this study is that of maintenance of performance level, where participants perform to what they feel is a "normal" level. The tasks used in this study generate an awareness of performance. Despite there being no active feedback, participants are aware of their general performance in that they know if they get a response correct or not. Given the changes detected in EFA levels in chapter 7, it may be that this maintenance of performance phenomenon exists independent of changes in EFA level. The changes in EFA level do affect performance in certain tasks, but not all of them.

Given these findings, one possible explanation is that participants baseline levels of EFAs were not low enough to induce performance deficits. In section 6.6, the levels of the sample population were found to be lower than dietary recommendations would recommend. While the hypothesis of low levels of EFAs in the sample population was supported by the blood levels of EFAs, it was also predicted that differences would exist in EFA levels between control and ADHD participants. However, the findings of section 6.6 found no such differences were detected.

It is possible that supplementation only is effective when EFA levels are extremely low. This has been demonstrated in animal models (Jensen, Skarsfeldt, & Høy, 1996). Jensen et al. (1996) studied three groups of rats, fed vegetable oils, marine oils or placebo. When the level of long-chain n-3 PUFA's was between 24% in the vegetable oil group and 27% as in the marine oil groups, no significant differences in performance on a spatial working memory task were detected. However, when these levels dropped to 22% as found in the control group, there were significant differences in performance. This provides evidence to suggest that while n-3 PUFA's were found to be associated with better cognitive performance, it wasn't until they dropped below a certain threshold that it appeared to have an effect on cognition. While an exact threshold has not yet been determined in humans, studies have provided evidence suggesting a similar

phenomenon. A study by Dalton et al. (2009) found that a fish oil supplementation in the form of a spread provided a beneficial effect on verbal learning and spelling in undernourished children (Dalton et al., 2009). Children received 25g a day of a spread either containing either fish-enhanced flour or a placebo on two slices of bread daily for a period of 6 months. These children were of a low socio-economic status, and had very low levels of EFAs supported by a reported low intake of fish in the diet. In contrary to this, numerous other studies conducted in properly nourished children have found no such differences. This provides further evidence for the suggestion that EFA supplementation may not be of benefit unless the levels have dropped below a certain level.

Another possibility is that omega-3 supplementation may only cause improvements in parameters that have an impaired performance to begin with. As this research was conducted on participants aged between 18 and 40, it could be argued that they should have a reasonably high baseline performance on cognitive tests when compared to an ageing population. While crystallised intelligence stays reasonably stable throughout the ageing process, fluid intelligence has been found to decline over the lifespan (Bugg et al., 2006). While this decline begins quite early while people are in their early to mid 20s, the process accelerates with age and is thought to begin before the age of 50 (Salthouse, 2004). Research has also found that self-reported difficulties in performance at the outset seems to correlate with the effectiveness of omega-3 supplementation (Jackson, Deary, et al., 2012). For example, different researchers have investigated the effects of n-3 supplementations in healthy adults. In one study, improvements in episodic memory and learning were reported in a sample of 437 participants who were supplemented with DHA over a 24 week period (Yurko-Mauro et al., 2010). In this study the selection criteria included self-reported memory problems and subjectively reported age related memory decline (Yurko-Mauro et al., 2010).

Data from this study may suggest the effects of these essential fatty acids on cognition and to differentiate the effects of DHA and EPA, but the small sample size restricts the validity of conclusions that are able to be drawn as the statistical power of the ANOVA drops relative to the sample size. Additionally, with a supplementation period of only 12 weeks it is difficult to draw valid findings from the study as the results may have been different if the participants were supplemented for a longer period. It is the recommendation that supplementation periods of longer than 12 weeks and larger sample sizes are used for future studies to further investigate the differences between the acids.

In summary, it appears that the maintenance of performance theory is consistent with the few major findings of this study. The participants are maintaining the performance they produced at baseline and this is acting independently of the effects of EFAs. In the case of more difficult cognitive tasks, some effects of EFAs were detected as well. It must be noted that this does not make any prediction in terms of changes in brain activity.

Chapter 9 Cardiovascular results

9.1 Abstract

In this chapter, the cardiovascular effects of the essential fatty acids used in this clinical trial are analysed. This chapter explored the effect of essential fatty acids on cardiovascular function and whether this changed based upon ADHD status and supplementation group. Cardiovascular parameters tested included peripheral and central blood pressure as well as common carotid blood flow velocity and arterial stiffness. Results suggest that there were few differences in cardiovascular function between control and ADHD participants after a 12-week supplementation period, with common carotid blood flow velocity being lower in Control participants. When supplementation data was analysed, Control and ADHD participants were combined as Chapter 6 showed no baseline differences between the groups. A between-groups by supplementation analysis found that EPA-rich supplementation caused a reduction in systolic and diastolic blood pressure while DHA appeared to affect only the common carotid blood velocity.

Blood Pressure

Previous research has established the effect of dietary factors such as omega-3 supplementation on lowering blood pressure (Kris-Etherton et al., 2002; Mori, 2010; Morris, Sacks, & Rosner, 1993), however the evidence is not as clear when looking at healthy individuals. A meta-analysis which identified a subset of eight studies using healthy participants with no clinical manifestation of disease found that the mean reduction in blood pressure for this group of studies was close to zero (Morris et al., 1993). The same meta-analysis suggested that there is a small but significant hypotensive effect in participants with existing hypertension (Morris et al., 1993). This is supported by other research suggesting that the extent of the hypotensive effects of omega-3s are dependent on the level of hypertension (Howe, 1997; Kris-Etherton et al., 2002) . This body of research suggests that there are beneficial effects of omega-3s on blood pressure, but these effects may be limited to people with existing blood pressure issues.

Arterial Stiffness

Previous research has suggested that arterial stiffness is a predictor of cardiovascular risk (Vlachopoulos et al., 2010; Wijendran, 2004). Arterial stiffness relates to the elasticity of the arterial walls, and their adaptability to adjust to pressure changes in the blood. Stiffer arteries have a reduced capacity to expand and contract in reaction to pressure changes

(Cecelja & Chowienczyk, 2012). Factors that can affect the stiffness of arteries include age (M. N. Levy & Pappano, 2007; O'Rourke, 2007) genetics (Lacolley et al., 2009) and lifestyle factors (Vlachopoulos et al., 2007). It has also been suggested that fish oil, with its high levels of omega-3 fatty acids, may be beneficial in reducing arterial stiffness with a recent meta-analysis suggesting that omega-3 fatty acids improve arterial stiffness (Pase et al., 2011). For these reasons, this study examined the effects of the supplementation of EFA on blood pressure to see if this differs between control versus ADHD participants and between supplementation groups.

Cerebral Blood Flow

Previous research has suggested that there is a link between essential fatty acid status and cardiovascular performance. Blood carries oxygen and nutrients to the brain and the brain needs substantial blood flow to the head in order to adequately function (Kandel et al., 2000). Despite only accounting for 2% of bodyweight, the brain consumes 15% of the cardiac output (Kandel et al., 2000). As the brain requires both oxygen and nutrients, this suggests that more blood flow and therefore more oxygen could arguably help the brain work more efficiently. While cerebral blood flow velocity does not measure volume directly, due to the blood oxygenation cycle a faster velocity means more blood is reaching the brain.

Aim

The aim of the present chapter was to investigate the effect that essential fatty acid supplementation has on cardiovascular functions in young adults both with and without ADHD.

Hypothesis

For the reasons outlined above, it was predicted that essential fatty acid supplementation would have a positive effect on cardiovascular health, with participants who were receiving EPA or DHA rich supplementation showing improvements in cerebral blood flow and arterial stiffness as compared to the placebo condition.

9.2 Methodology

Participants

The sample population was as reported in the previous chapters of the thesis. It consisted of 98 participants aged between 18 and 40 years of age, consisting of a control group of

participants and a group of participants who suffered from ADHD. The control participants (40 males and 20 females) were aged between 18 and 40 years (M=24.8 years, SD=4.94 years). The participants who suffered from ADHD (19 males and 19 females) were aged between 18 and 36 years (M=24.97 years, SD=4.76 years). All participants had normal or corrected to normal vision, no colour blindness and were free from neurological conditions.

Experimental design

The present study adopted a double blinded, randomised, placebo-controlled parallel design. For details on study design and treatment randomisation refer to section 5.3.

9.3 Results

Differences Between Control/ADHD Baseline

Results presented earlier in the thesis showed that the cardiovascular performance of ADHD participants and control participants were comparable at baseline, with no differences detected in the majority of variables. The only significant difference that was detected at baseline was in the central pulse pressure, with control participants having an average higher central pulse pressure reading than ADHD participants. These results and corresponding figures were presented in Table 6-6.

After Supplementation- Between Groups - ADHD and Control

After the baseline levels of cardiovascular function were tested, an ANOVA was conducted to detect the differences in cardiovascular function after the supplementation period.

In order to analyse cardiovascular function after supplementation across the control and ADHD participants, a series of independent groups t-tests were conducted. Results are presented below in Table 9-1.

Table 9-1: Mean values for cardiovascular variables across control and ADHD groups after supplementation.

	Control		ADHD				
	Mean	SE	Mean	SE	f	df	p
Peripheral systolic pressure (mmHg)	120.2	2.3	123.0	3.4	-0.6	62	0.57

Peripheral diastolic pressure (mmHg)	73.5	1.6	77.6	2.7	-1.4	62	0.18
Peripheral pulse pressure (mmHg)	46.7	2.0	45.2	2.3	0.4	61	0.67
Central systolic pressure (mmHg)	105.5	2.0	106.1	2.8	-0.2	61	0.87
Central diastolic pressure (mmHg)	74.5	1.6	77.7	2.7	-1.0	61	0.30
Central pulse pressure (mmHg)	31.0	1.50	28.4	1.2	1.4	59	0.18
Central augmentation index	115.7	2.0	111.3	3.6	1.1	61	0.26
Average common carotid blood flow (cm/sec)	22.1	0.6	24.2	0.9	-2.0	74	0.05

*p<.05, **p<.01

This is also shown graphically in Figure 9-1 below.

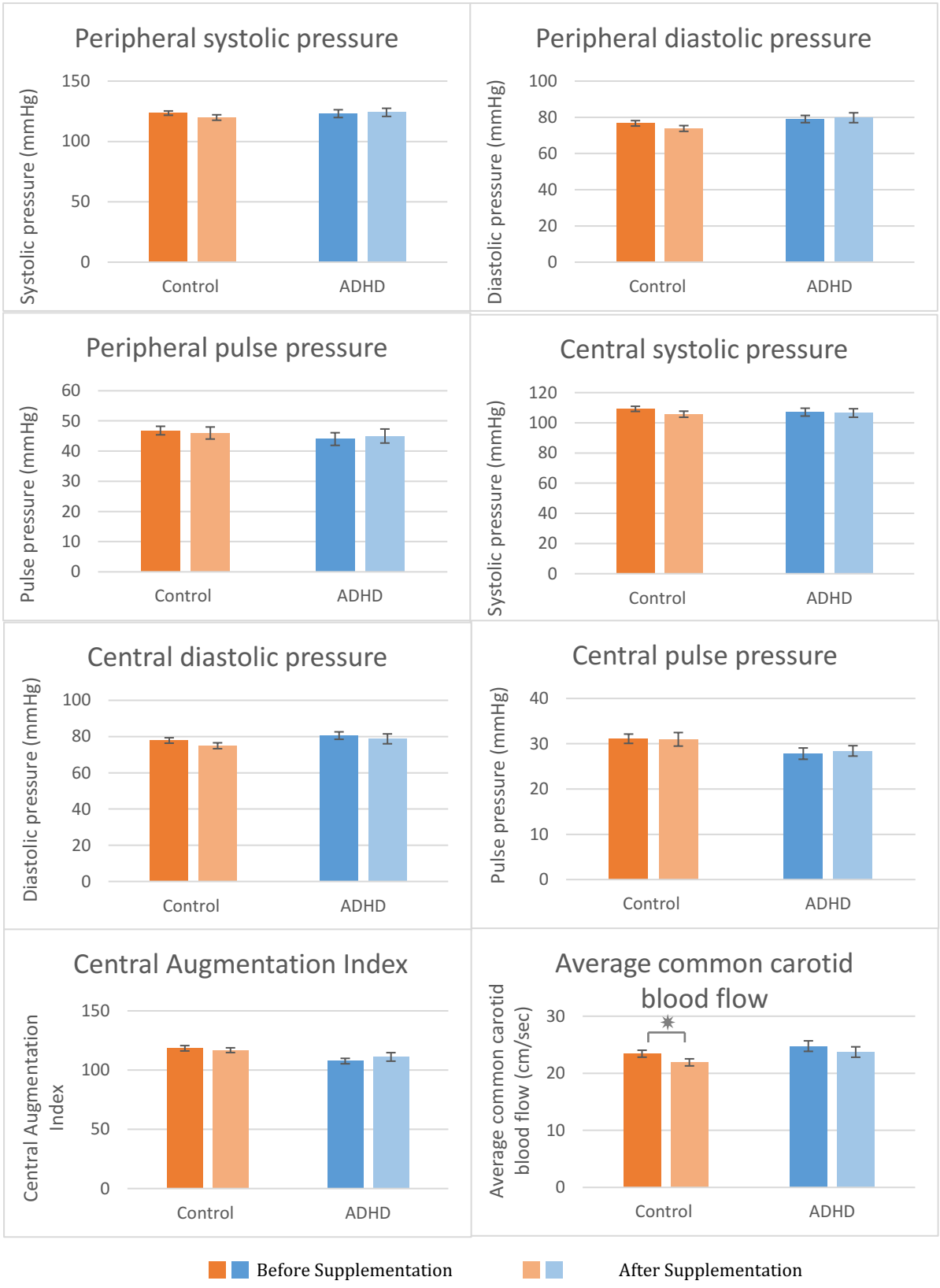


Figure 9-1: Baseline and after supplementation cardiovascular function by control and ADHD (*p<.05, **p<.01).

While the tables and graphs above show there were no significant differences between control and ADHD participants after supplementation, blood flow velocity was extremely

close to reaching statistical significance with a p value of 0.05. When the individual groups were investigated control participants did show a significant difference in blood flow velocity between the time points, however this result must be interpreted with caution. It is close to the level of significance, and as it is the only significant result out of 16 comparisons, it is possibly due to chance.

Within Groups - ADHD and Control

While there were no significant differences between control and ADHD participants after supplementation, when looking at within group comparisons, a significant difference was detected in common carotid blood flow velocity between the two time points for control participants. This can be seen below in Table 9-2, and is also indicated in Figure 9-1 above.

Table 9-2: Summary of baseline and after supplementation cardiovascular differences between Control and ADHD participants.

	Control	ADHD
Peripheral systolic pressure	NS	NS
Peripheral diastolic pressure	NS	NS
Peripheral pulse pressure	NS	NS
Central systolic pressure	NS	NS
Central diastolic pressure	NS	NS
Central pulse pressure	NS	NS
Central augmentation index	NS	NS
Average Common carotid blood flow	✓	NS

✓: $p < 0.05$, ✓✓: $p < 0.01$, NS: $p >= 0.05$

When the effects of baseline levels of EFA on cardiovascular performance were investigated in section 6.7, it was determined that there were very few differences detected between control and ADHD participants on cardiovascular variables. The only difference that was detected was in central pulse pressure, where control participants were found to have slightly higher average values. This result must be interpreted with caution however, due to the marginality of the p-value detected. Due to the lack of difference, it was deemed appropriate to compare the population as a whole (ADHD and

control) in terms of supplementation group as was done in Chapter 8, improving statistical sensitivity. This gives an indication of the cardiovascular effects of the acids independently of ADHD status, but by increasing the sample population size, it increases the statistical power of the study.

Differences Between Supplementation groups at baseline

Below are tables looking at the differences between the supplementation groups at baseline. In order to analyse the differences in cardiovascular function between supplementation groups at baseline, a one-way analysis of variance was conducted. Results are presented below in Table 9-3.

Table 9-3: Mean values for cardiovascular variables across supplementation groups at baseline.

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	f	df	p
Peripheral systolic pressure (mmHg)	127.9	2.9	126.4	3.1	120.3	2.2	2.1	2	0.13
Peripheral diastolic pressure (mmHg)	80.8	2.2	78.8	2.3	76.1	1.7	1.2	2	0.29
Peripheral pulse pressure (mmHg)	47.2	2.0	47.6	2.3	44.2	1.8	0.02	2	0.98
Central systolic pressure (mmHg)	112.1	2.7	111.6	2.6	105.5	2.0	2.2	2	0.12
Central diastolic pressure (mmHg)	82.1	2.2	80.2	2.3	77.2	1.7	1.4	2	0.26
Central pulse pressure (mmHg)	30.0	1.4	31.4	1.4	28.3	1.4	1.2	2	0.32
Central augmentation index	112.0	2.8	117.9	3.2	112.0	2.6	1.4	2	0.26
Average common carotid blood flow (cm/sec)	22.5	0.8	22.7	1.0	25.1	1.0	2.6	2	0.08

p<.05, **p<.01

At baseline, no significant difference was detected between the three supplementation groups for any of the cardiovascular variables measured. This can also be seen graphically below in Figure 9-2.

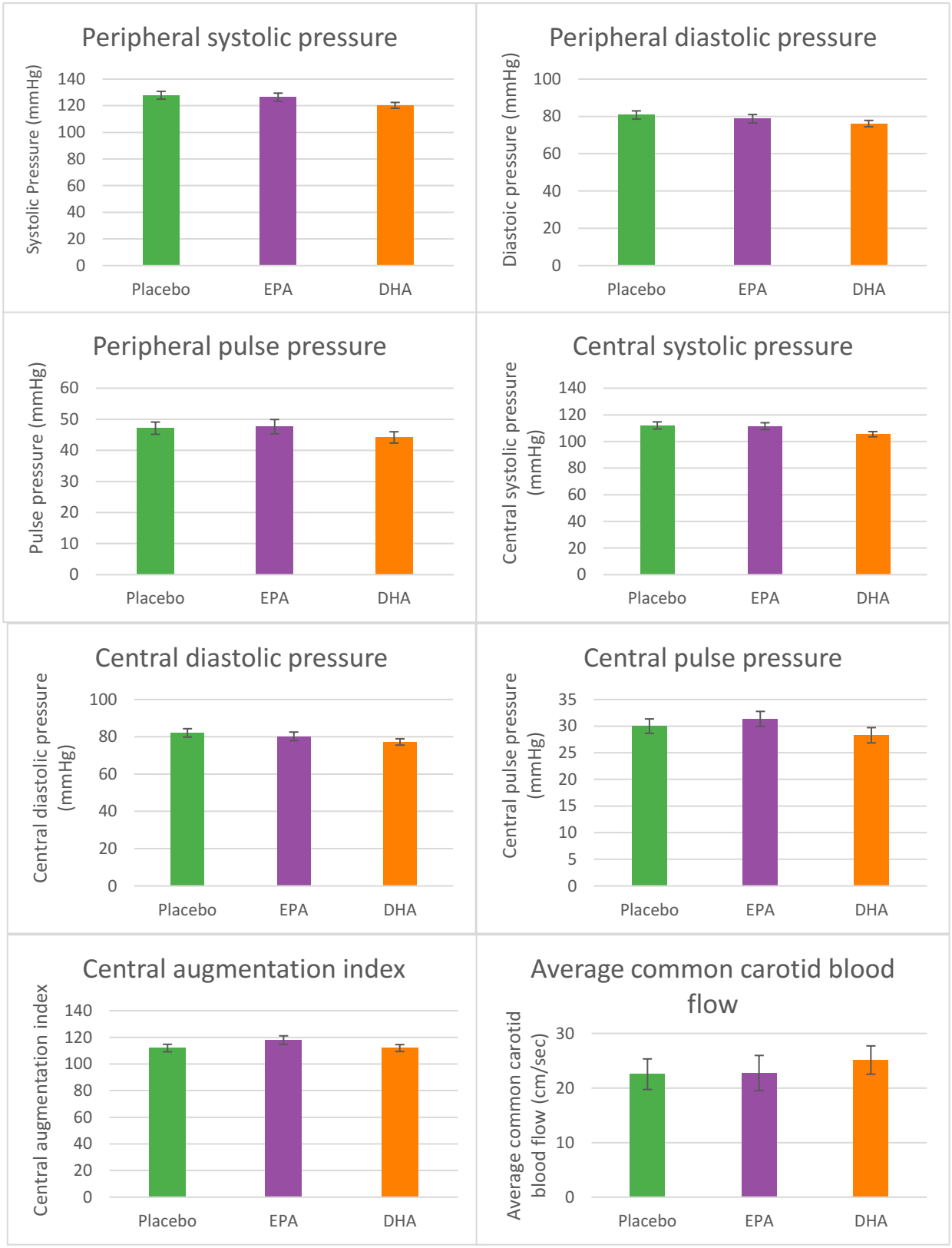


Figure 9-2: Baseline cardiovascular performance by supplementation group.

Between Groups (Supplementation)

In order to analyse cardiovascular function after supplementation across the supplementation groups, a series of one-way ANOVAs were conducted. Results are presented in Table 9-4 and Figure 9-3.

Table 9-4: Mean values for cardiovascular variables across Control and ADHD groups after supplementation.

	Placebo		EPA-rich		DHA-rich				
	Mean	SE	Mean	SE	Mean	SE	f	df	p
Peripheral systolic pressure (mmHg)	121.0	3.1	118.4	3.5	122.9	3.2	0.5	2	0.64
Peripheral diastolic pressure (mmHg)	74.1	2.2	72.0	2.1	78.1	2.8	1.6	2	0.22
Peripheral pulse pressure (mmHg)	46.9	2.4	46.4	3.0	45.2	2.9	0.1	2	0.90
Central systolic pressure (mmHg)	105.4	2.6	104.3	3.3	107.4	2.8	0.3	2	0.76
Central diastolic pressure (mmHg)	75.1	2.2	73.1	2.2	78.3	2.9	1.1	2	0.34
Central pulse pressure (mmHg)	30.3	1.5	31.2	2.2	29.1	2.2	0.3	2	0.76
Central augmentation index	112.2	3.0	117.8	2.9	113.6	3.3	0.9	2	0.42
Average common carotid blood flow (cm/sec)	23.3	0.87	22.8	0.8	22.2	1.1	0.4	2	0.66

*p<.05, **p<.01

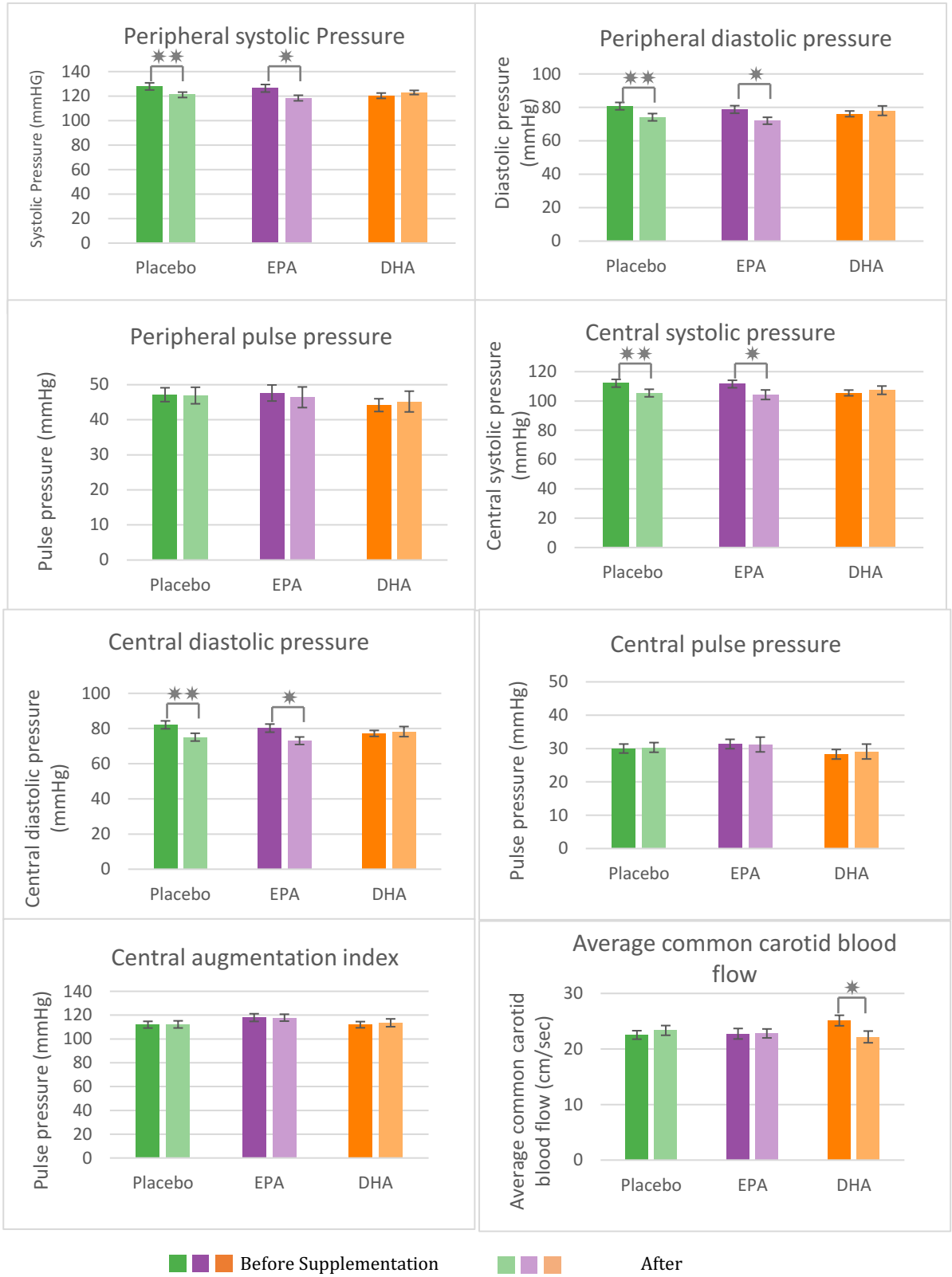


Figure 9-3: Baseline and after supplementation cardiovascular function by supplementation group.

Within Groups – Supplementation groups

While there were no significant differences between control and ADHD participants after supplementation, when looking at within group comparisons, a significant difference was detected in common carotid blood flow velocity between the two time points for control participants. This can be seen below in Table 9-2, and is also indicated in Figure 9-1 above.

Table 9-5: Summary of baseline and after supplementation cardiovascular differences between supplementation groups.

	Supplementation Group		
	Placebo	EPA-rich	DHA-rich
Peripheral systolic pressure	✓✓	✓	NS
Peripheral diastolic pressure	✓✓	✓	NS
Peripheral pulse pressure	NS	NS	NS
Central systolic pressure	✓✓	✓	NS
Central diastolic pressure	✓✓	✓	NS
Central pulse pressure	NS	NS	NS
Central augmentation index	NS	NS	NS
Average common carotid blood flow	NS	NS	✓

✓: $p < 0.05$, ✓✓: $p < 0.01$, NS: $p >= 0.05$

9.4 Discussion

The current study explored the cardiovascular effects of a 12-week EFA supplementation on control participants and participants who suffered from ADHD. Based on previous research(Fontani et al., 2005; Kidd, 2007) , it was predicted that EFA supplementation would improve cardiovascular performance as measured by decreased blood pressure and increased cerebral blood flow velocity.

Control and ADHD

Contrary to the hypothesis, there were no significant differences between control and ADHD participants after supplementation for the cardiovascular variables measured. This hypothesis was based on previous research suggesting that differences exist between

control and ADHD participants in terms of EFA status. As EFA status has been found to be related to cardiovascular health, it stands to reason that if a particular group has lower levels of EFAs then they might have poorer levels of cardiovascular performance. However, earlier in this thesis, results showed there were no differences in EFA levels between control and ADHD participants in this particular sample. This could explain why there were minimal cardiovascular differences detected between the groups in this sample.

Supplementation Groups

At baseline, no significant differences were detected between the three supplementation groups for any of the cardiovascular variables measured. This is beneficial for the study, as provides evidence for the validity of after supplementation comparison. If the groups had differed at baseline, then this would have had to be taken into account when conducting the after supplementation comparisons.

In contrast to the hypothesis, no differences were detected between the supplementation groups after supplementation. Perhaps more interesting though, is the differences pre and post supplementation amongst the groups. Both the placebo and EPA-rich supplementation had significant decreases in systolic and diastolic pressure over the supplementation period. This pattern is identical for both peripheral and central measures of blood pressure and this helps to strengthen the validity of these results. The DHA-rich supplementation group did not show any changes in systolic or diastolic blood pressure, either centrally or peripherally over the same period.

The results above imply that the placebo and EPA-rich supplementation have an effect on blood pressure where the DHA-rich did not. This is of interest, as it was not expected and brings up the question of the suitability of the placebo treatment used. The placebo treatment used was a mix of omega-6 and omega-9 PUFAs.

One important question that must be considered when examining these results is what is meant by improved cardiovascular function. In terms of cerebral blood flow velocity, research has found that reduced blood flow to the temporoparietal cortex can be an early sign of Alzheimer's (Suo et al., 1998) and that hypo perfusion can also be a risk factor for other health issues including stroke (O. Y. Bang et al., 2008). However, care must be taken before drawing assumptions, as just because reduced blood flow is detrimental to health, it does not necessarily imply that increased blood flow has a positive impact on health. More research needs to be conducted in order to establish this using empirical research. Additionally, even if it is assumed that increased blood flow is a good thing, the nature of

this relationship needs to established determining whether the increased blood flow is a direct result of the increased EFA level or whether it may work through other possible mechanisms such as inflammation or membrane fluidity.

Conclusion

In summary, the major findings of this study suggest that there were few differences in cardiovascular function between the supplementation groups at baseline. Similarly, there were few differences detected at baseline between the Control and ADHD participants. An interesting pattern of supplementation effect was observed involving a reduction in blood pressure for the EPA rich supplementation but not for the DHA-rich group, and similarly, a reduction in common carotid blood flow velocity in the DHA group, while no change in velocity was detected in the EPA-rich group.

Chapter 10 fMRI results

10.1 Abstract

This chapter explores the effect of essential fatty acids on brain activation using fMRI analysis. A 3-condition modified colour-word Stroop task was used as a measure of cognitive interference, investigating differences in brain activation based upon ADHD status and supplementation group. A region of interest (ROI) based approach generated a target network including the anterior cingulate cortex, the inferior frontal junction and the right medial frontal gyrus, comparing activations for Congruent, Incongruent and Advanced Incongruent tasks. In several ROIs of the network, the ADHD group showed more sustained activation than did the Control group. Also, those who received DHA-rich supplementation showed sustained levels of activation compared with those who received EPA-rich supplementation.

10.2 Introduction

fMRI technique

In 1990, Ogawa and colleagues discovered that the magnetic properties of haemoglobin change depending on whether it is oxygenated or not (Ogawa, Lee, Nayak, & Glynn, 1990). This shift in magnetic signal, detected by the MRI scanner, resulted in the technique known as functional magnetic resonance imaging (fMRI). With ultra-high field magnets, activation across the human brain can be determined with excellent spatial resolution of approximately one millimetre.

The fMRI technique relies on the blood oxygen level dependent (BOLD) response. As neurons do not store large quantities of energy supplies such as sugar or oxygen, they rely on the blood system to respond to energy demand by a local increase in blood flow. An increase in the fMRI signal results from an increase in the ratio of diamagnetic oxygenated haemoglobin (oxyHb) relative to paramagnetic deoxygenated haemoglobin (deoxyHb). The fMRI signal (BOLD response) depends on the increase in oxygenated blood flowing into an area indicating a change in neural activity, with this indirect measure demonstrating a temporal lag of a few seconds. If certain areas of the brain show changes in activation during the completion of a cognitive task, these regions are then deemed involved in this task. Because the whole brain, when divided into cubes commonly 3mm on edge, contains thousands of voxels, new statistical techniques have had to be developed to handle the issue of multiple comparisons. These include cluster analysis, on the basis that the probability of neighbouring voxels should show activity together is less likely to be by chance. False discovery rate (Genovese, Lazar, & Nichols, 2002) estimates

the likelihood that natural signal fluctuations should generate significant voxels. The ROI-based approach used in this thesis reduces the multiple comparison problem. It does this by identifying the regions of interest and applying corrections separately within each region (Genovese et al., 2002). fMRI can be seen as an indirect measure of neural activity, as it measures the magnetic response to blood flow generated by neural activity rather than the activity itself (Arthurs & Boniface, 2002).

10.3 Stroop Task, fMRI and ADHD

Selective attention plays a key role in the ability of people to complete cognitive tasks that require dealing with cognitive interference. The Stroop task (Stroop, 1935) is a widely used test of cognitive interference. It asks participants to inhibit their normal response by completing a colour-naming task which uses a decision rule requiring the normal behaviour to be inhibited (Leung, Skudlarski, Gatenby, Peterson, & Gore, 2000). For example, a participant would typically be slower in naming the ink colour of the word "*blue"* that is printed in red ink than naming *congruent colour pairs (ie **Blue** versus **Blue**).* This is due to the cognitive inhibition required to correctly complete the task (Leung et al., 2000).

Attentional deficits are one of the key symptoms of ADHD (see section 4.3 of this thesis) and poor inhibitory control is a central feature of impulsiveness and is commonly observed to be impaired in ADHD (Paloyelis, Mehta, Kuntsi, & Asherson, 2007; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). As such, the Stroop task has been used in previous research (Godinez et al., 2015; Hwang et al., 2015; Kim, Sharma, & Ryan, 2015) for differentiating brain activation patterns between ADHD participants and controls.

The use of fMRI in the investigation of ADHD is a rapidly developing field of research. The majority of studies adopt a task-based approach, examining differences in task activation for groups of ADHD versus Control participants (Paloyelis et al., 2007). A commonly adopted task design is the block design, where activation difference signals are obtained through subtracting brains activation levels from blocks recorded in an 'off or rest' condition from blocks recorded in an 'on or task' condition. Multiple studies have investigated fMRI activation levels while completing the Stroop task. The congruent condition of the task has been associated with increased BOLD levels in the inferior frontal cortex and the incongruent condition being associated with dorsolateral prefrontal areas (Carter et al., 2000; Evers, Van der Veen, Jolles, Deutz, & Schmitt, 2006). Carter et al. (2000) found greater activation in the anterior cingulate cortex (ACC) for tasks involving conflict, with the authors suggesting that the ACC is involved with an

evaluative function, reflecting the degree of response conflict elicited by the task (Carter et al., 2000). Bush et al. (1999) studied 16 subjects (8 control and 8 unmedicated ADHD). Using a counting Stroop task, results showed significant activation of the ACC region of the brain in the control participants, but not in ADHD participants. This was found to be task related, as no difference in ACC activation was detected when comparing group responses to a neutral task. (Bush et al., 1999).

Bauer et al. (2014), using a cross-over study investigated the effects of EPA-rich and DHA-rich supplementation on cognitive performance and functional brain activation in a neurotypical population. The authors concluded that EPA-rich supplementation improved neural efficiency and cognitive performance in a young adult population. This was demonstrated by a reduction in functional brain activation in the left anterior cingulate cortex along with an improvement in cognitive performance. This pattern of results was not observed following DHA-rich supplementation. However, it must be noted that the periods of supplementation (30 days) were relatively short.

While there are many similarities between the two studies, there are also key differences. The most obvious difference lies in the experimental design. The Bauer at al study was a cross-over design, whereas the current study was a parallel group design. Additionally, the current study has a placebo arm, whereas Bauer et al. (2014) did not. The second major difference is that the Bauer et al. study used supplementations that were off-the-shelf ADHD treatments, either Eye-Q (EPA-rich) or Efalex (DHA-rich) - these preparations also containing other lipids, such as oil of primrose (Eye-Q) and D-alpha-tocopherol, evening primrose oil and thyme oil (Efamol). In addition, the supplementation period was relatively short – 4 weeks, compared with the current study of 12 weeks supplementation. Finally, the study was grounded in a normal population rather than a comparison of Controls versus ADHD as is reported here.

ROIs

The approach taken in this study of the effects of omega-3 oils on brain activity was to select specific regions of interest (ROIs), guided by previous fMRI research on tasks similar to those undertaken here. As discussed in Poldrack (2007), there are many reasons why ROI analysis is undertaken. The reason in this study was for statistical control. Before the analysis was undertaken, a set of anatomical ROIS were selected. This is done so when correction is undertaken for multiple comparisons, this is only undertaken for the ROIs selected rather than the large number of voxels in the entire brain (Poldrack, 2007). For this particular study, the anterior cingulate cortex (ACC) was chosen as the main region of interest for the Stroop task. While there are likely global

brain effects as a result of ADHD, this particular area was chosen as it is likely to be activated during the completion of the Stroop task (Bauer et al., 2014; Bush et al., 1999; Dramsdahl, Westerhausen, Haavik, Hugdahl, & Plessen, 2011; Van Veen, Cohen, Botvinick, Stenger, & Carter, 2001). The ACC is a brain area associated with cognitive control and attention (Makris et al., 2010) thus any anatomical or functional differences in this area may relate to the ADHD symptoms of impulsivity, hyperactivity and inattention. Other ROIs (Inferior frontal junction, Left inferior parietal lobe, Left medial frontal gyrus, Left precuneus, Right medial frontal gyrus) were also selected from activations found in the above studies. As inattention is the most apparent symptom of ADHD, then it stands to reason that brain areas associated with attention would have different activation when performing attentional tasks in participants with ADHD in comparison to control participants.

Neural Efficiency

The level of effort required in a cognitive task can affect the corresponding brain activation, and this is related to behavioural performance. When attempting to evaluate the relationship between neural activity and cognitive performance, there needs to be an underlying theory that can integrate the two measures. One such theory is the theory of neural efficiency, a theory that can connect the tasks performance and the neural activation that accompanies that behaviour. The theory of neural efficiency was developed by Haier et al. (1988) in order to explain the inverse correlation between Ravens' score and glucose metabolic activity in a group of young adults aged between 18 and 30. This finding was replicated by further research which also observed an inverse relationship between IQ and glucose metabolic rate (Haier, Siegel, Tang, Abel, & Buchsbaum, 1992). Based on these findings, the authors hypothesised that individuals with a higher IQ may require less neural resources when undertaking higher order cognitive processes than that of an individual with a lower IQ (Haier et al., 1992; Haier et al., 1988). This is the theory of neural efficiency which states that differences in human cognitive ability are not a function of how hard the brain is working, but rather the efficiency of the brain processes that are being undertaken (Haier et al., 1988). This can be interpreted to mean that "smart brains work less hard" in order to undertake the same task.

This theory has since been illustrated using different methodologies. EEG studies have found a relationship between cognitive performance and the amplitude of the alpha to theta frequency band, with lower amplitudes in these bands being associated with better

cognitive performance (Doppelmayr, Klimesch, Schwaiger, Auinger, & Winkler, 1998; Klimesch, 1999).

10.4 fMRI research and omega-3 fatty acids

Research into the effects of omega-3 FAs on cognitive performance in young adults is limited (as reviewed in Chapter 3.2), with a majority of supplementation studies using DHA. Thus, there is insufficient research directly comparing the cognitive and brain effects of EPA and DHA, and the existing literature is equivocal in indicating relative effects. Jackson, Reay, Scholey, and Kennedy (2012) in a parallel design found that neither DHA or EPA supplementation led to a significant reduction in simple or choice reaction times. In contrast to this finding, Bauer et al. (2011), in a cross-over design, found EPA supplementation led to faster choice reaction times when compared to DHA-rich supplementation on a psychophysiological task. These contrasting findings highlight the need for further research in this area to determine whether the different effects of EPA and DHA and which, if any, of these has a greater effect on cognitive performance.

The task chosen for this investigation was a version of the Stroop task with three different conditions: Congruent, Incongruent and Advanced Incongruent (see chapter 5.7 for details). This task tests the effect of interference on reaction time, requires focussed attention, and is associated with cognitive control. This makes it a relevant task to use when looking at activation of the ACC, and it has been used in numerous other studies on an ADHD population (Bledsoe, Semrud-Clikeman, & Pliszka, 2013; Ikeda, Okuzumi, Kokubun, & Haishi, 2011; Mercadilo, Trujilo, Sánchez-Cortazar, & Barios, 2012).

Aims

There were two aims of this chapter. The first aim was to examine whether there was any difference in brain activation between the ADHD and the control participants at baseline. The second aim of this chapter was to examine the effects of 12 weeks EFA supplementation on neural function during higher order cognitive tasks.

Hypotheses

Based on previous research, it was predicted that there would be differences in activation of brain areas associated with Stroop task performance, between the Control and ADHD participants, in areas of the brain associated with symptoms of ADHD, such as attention and executive function. More specifically, it was predicted that there would be differences in activation observed in the ACC, as it is a brain area that is related to attention and cognitive control, and it has previously been shown to be activated during performance of the Stroop task.

It was also predicted, on the basis of the theory of neural efficiency, that 12 weeks of EFA supplementation would lead to a reduction in functional activation relative to performance when the participants were performing higher order cognitive tasks. It was also predicted that the EPA supplementation would have a greater effect on activation differences than the DHA supplementation.

10.5 Method

Two participant groups, ADHD and Control, were tested in a parallel design on the 3-legged Stroop task at baseline and following a 12-week supplementation period. Participants were randomly allocated to one of three treatment groups (Placebo, EPA or DHA), with treatments detailed in section 5.3.

10.6 Supplementation

Participants were instructed to take four capsules per day with food, and were advised not to consume more than the recommended dosage in order to avoid potential side effects of the capsules and to ensure all participants received the same dosage. The study used three randomly assigned formulations, either an EPA-rich formulation with a 4:1 EPA to DHA ratio, a DHA-rich formulation with a 4:1 DHA to EPA ratio, or a placebo formulation primarily consisting of soya oil and free of both EPA and DHA. All formulations were identical in appearance. For details on the ingredients of the supplementation see Table 5-2 and Table 5-3 in Chapter 5 of this thesis.

10.7 Participants

All fMRI participants were also part of the larger study, detailed in chapters 6 to 9. The fMRI participants were selected through their availability, eligibility and willingness to participate. All participants were given the option of completing the fMRI aspect of the study. For inclusion and exclusion criteria and recruitment methods, see sections 5.4 and 5.5. All participants signed an informed consent form, in accordance with the Swinburne University Human Research Ethics Committee. At baseline, fifty-five participants were tested using fMRI. Thirty of these were from the Control participants and twenty-five were from the ADHD group. Demographic details for these participants can be found below in Table 10-1. After the supplementation period, twenty-six control participants and twenty participants with ADHD symptoms completed the fMRI scan. This participant dropout, of roughly 20% was not biased to either Controls or ADHD and was largely due to time pressures later in the year, leading to unwillingness to complete another fMRI scan.

10.8 Testing procedure

Participants attended two 3-hour testing sessions at Swinburne University, Melbourne, Australia. After reading and signing the information consent form, participants completed a demographic questionnaire. They performed the SUCCAB cognitive assessment battery (Pipingas et al., 2008). For the methodology of the SUCCAB, see chapter 5.7 and for the results see chapter 8.4 of this thesis. Participants underwent two fMRI brain scans at the Brain Research Centre (Austin Hospital, Heidelberg, Australia) 12 weeks apart. Supplementations were provided at the end of the first testing session.

Colour–Word Stroop Test

Outside the scanner, the Colour–Word Stroop task was presented on 17-in. colour CRT monitor using a DOS-based computer software program. This seemingly archaic system was selected as it allowed for millisecond timing precision of keyboard responses. Participants had a practice run through of the task so they could familiarise themselves with the task before the experiment began, and they had an opportunity to ask questions prior to beginning the tasks.

In the colour–word Stroop task, participants were presented with names of colours (red, blue, green and yellow) and were instructed to respond by pressing the button of the corresponding colour of the pixels. The task included a Congruent condition, an Incongruent condition and an Advanced Incongruent condition. In both the Congruent and Incongruent tasks, the participant was asked to push the button that corresponded to the colour of the text as quickly as possible.

In the Congruent condition, the words red, blue, green and yellow were presented in the matching text colour with no interference. In the Incongruent condition, the same words were presented in red, blue, green or yellow text, but in this condition the colour of the text did not match the written word. For example, the word “Red” was written in blue, green or yellow coloured pixels. In the Advanced Incongruent condition, all of the stimulus words were presented in incongruent colours. 50% of the stimuli (presented in random order) were surrounded by a black rectangle. In this circumstance, the participant was instructed to press the button whose colour corresponded to the **meaning** of the word. If the stimulus word was presented without a surrounding rectangle, the participant was instructed to press the button that corresponded with the **colour** of the pixels.

The Stroop interference index was calculated by subtracting reaction times on correct trials for the Congruent condition from reaction times for the Advanced Incongruent condition.

Imaging procedure

Participants who took part in the fMRI part of the study each underwent two scans, one at baseline and one after the 12-week supplementation period. Scans were conducted at the Brain Research Institute in Heidelberg, Melbourne, Australia and were conducted using a 3 Tesla Siemens Tim Trio MRI scanner (Siemens, Erlangen, Germany) equipped with a 12-channel head coil. At the beginning of the baseline session, a high resolution T1 weighted image was acquired (coronal slice acquisition). This used a 3D MPRAGE sequence (TR = 1900 ms, TE = 2.6 ms, 192 slices, 0.9x0.9x0.9 mm voxels, FOV 230 mm, slice thickness 0.9 mm). For the testing session conducted after supplementation, 66 functional images were obtained using a T2 weighted gradient echo-planar pulse sequence (TR = 3000, TE = 30 ms, FOV = 216 mm, voxel size 3 x 3 x 3 mm).

Upon arrival at the scanning facility, each participant undertook a safety screening procedure. This was in addition to the phone screening procedure conducted at time of recruitment and was conducted by a qualified radiologist. After this, the participant removed all metal (jewellery, electronic devices etc.) from their body and entered the scanner in a supine position. For the duration of the scan, participants were asked to stay as still as possible in order to minimise head movement. Foam padding was placed around the participant's neck to assist this. During scanning the participant was in contact with the researchers and the MRI technician via a MRI-compatible microphone. Stimuli for the task were presented on a MRI-compatible screen located behind the scanner, viewed by the participants with the assistance of a mirror.

Imaging analyses

Pre-processing and statistical analyses were performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK). Before pre-processing commenced, the first six volumes were discarded from each functional sequence to reduce T1 saturation effects in image time-series. The "ArtRepair" tool was then used to clean voxels and repair slices with high variance levels. These corrected images were realigned to the first image of the first session creating a mean realigned image. The T1-weighted structural image was then co-registered to the mean re-aligned image that had been created. This was visually checked and spatially normalised to the template provided by SPM8. This T1 spatial normalisation was applied to the realigned echo planar images and these were smoothed

through the use of a Gaussian kernel. ArtRepair was then used to detect and repair any volumes which still exhibited high variation in signal intensity.

During the fMRI investigation, for each scanning run of the colour–word Stroop task 60 volumes were analysed. This involved four active blocks: two 24-s blocks of Congruent stimuli and two 36-s blocks of Incongruent stimuli. Congruent blocks contained 12 stimuli (trials), whereas Incongruent blocks contained 18 stimuli (trials), with rest periods (no stimuli) interspersed. For all conditions, stimuli were presented for 1000 ms followed by a 2000-ms black fixation cross on a white screen.

Two levels of analysis were employed: at the first level, the fMRI data (after preprocessing) were modelled together across sessions for each individual, and were modelled for each testing session (2 participant groups x 3 diets) using the same parameters. The time sequence of scans was high-pass filtered and a multiple regression model was applied. Box-car regressors (for task but not resting periods) were convolved with the hemodynamic function (SPM8). The effect of head motion during the scans was taken into account by adding the Cartesian motion (X,Y,Z) and rotation parameters (yaw, pitch, roll) to the statistical model as regressors of no interest for each testing session. Estimates of the beta parameters derived from the general linear model as mean slopes of the explanatory vectors of the regression model, for each task condition (Congruent Stroop, Incongruent Stroop, Advanced Incongruent Stroop), were computed at baseline and after supplementation.

The contrast maps for the larger model representing the difference between the beta parameter estimates over the three sessions were entered into a one-sample t-test. Statistical thresholding for the resultant group activation map was $p < .001$ (uncorrected) at the voxel level, and only those clusters that were significant after correcting for multiple comparisons ($p \leq .05$, FWE corrected) at the cluster level were considered significant.

These clusters were used as the basis for a regions of interest (ROI) analysis that was performed using the MarsBaR Region of Interest toolbox for SPM (Brett, Anton, Valabregue, & Poline, 2002) to compare the effects of Placebo, EPA-rich and DHA-rich supplementations on functional activation. Initially, a sphere of 10 mm radius was constructed around the peak coordinates of significant activation clusters. Then, the mean contrast at each time point within each ROI was extracted from each session-specific contrast map, yielding 6 values per ROI for each participant corresponding to Placebo, EPA-rich, and DHA-rich for the Control group and for the ADHD group.

10.9 Results

fMRI Demographics

In order to analyse the differences in demographic variables between control participants and ADHD participants who completed the fMRI at baseline, an independent groups t-test was conducted. Participant demographics for the control participants and the participants with ADHD are shown in Table 10.1 below.

Table 10-1: Demographic data of fMRI participants by Control/ADHD.

	Control	ADHD	t	p
Number of participants	30	25		
Gender (Males %/Females %)	73/27	48/52	-1.96	0.05
Age	24.56(4.62)	24.72 (4.95)	-0.12	0.91
Height (cm)	174.73 (9.24)	173.07(11.74)	0.56	0.58
Weight (kg)	72.33(11.92)	71.88 (15.13)	0.12	0.90
Body Mass Index (BMI)	23.84(3.29)	24.03(4.93)	-0.16	0.87
Education Level[8]	3.43(1.07)	2.68(.84)	2.73	0.01*
Exercise[9]	3.39 (1.10)	3.52(1.54)	-0.35	0.73
Smoking status[10]	1.48 (0.51)	1.45(0.67)	0.17	0.87
Fish Intake[11]	2.53(1.04)	2.52(0.99)	0.04	0.97
Fruit and vegetable intake[12]	1.90 (.88)	2.04 (0.98)	-0.56	0.58
Junk food intake[13]	3.27 (0.64)	3.30 (0.88)	-0.18	0.86

[8] 1=Primary school,2=Secondary school,3=TAFE,4=Undergraduate degree, 5=Postgraduate degree. See Appendix II for copy of questionnaire

[9] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week, 5=Daily See Appendix II for copy of questionnaire

[10] 1=Yes, 2=No See Appendix II for copy of questionnaire

[11] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire

[12] 1=Several times a day, 2=Once a day,3=2-3 times a week,4=Once a week,4=Very rarely See Appendix II for copy of questionnaire

[13] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire

Takeaway intake[14]	3.07 (0.91)	3.08(0.95)	-0.08	0.94

*p<.05, **p<.01

Table 10-1 shows that the Education level of the ADHD group was significantly less than Controls with much greater tertiary education experience in the latter group. The gender balance was also marginally different, with a bias towards females in the Control group and near equality in the ADHD group. The bias towards females for the Control group likely reflects Psychology based courses as the major recruiting ground for Controls, whereas ADHD participants were recruited through other channels.

The data were initially normalised by minimising the initial differences between group activations (every graph starts at zero). This condition comparison was chosen for analysis because it compares the activation for the easiest version of the task versus the most difficult version of the task (validated by the behavioural results of Chapter 8). This is the most likely comparison to bring out differences between the participants. This is evidenced from section 8.4 showing the largest difference in behavioural results being between the Congruent and Advanced Incongruent conditions.

fMRI analysis of ADHD versus Control

In order to detect any differences in brain activation between Controls and ADHD, a series of t-tests were conducted on ROI activations over trial duration using an event-related design. The activation analysed in detail was the Advanced Incongruent activation minus the Congruent activation on the same task.

[14] 1=Never, 2=Once a month, 3=Once a week, 4= More than once a week See Appendix II for copy of questionnaire

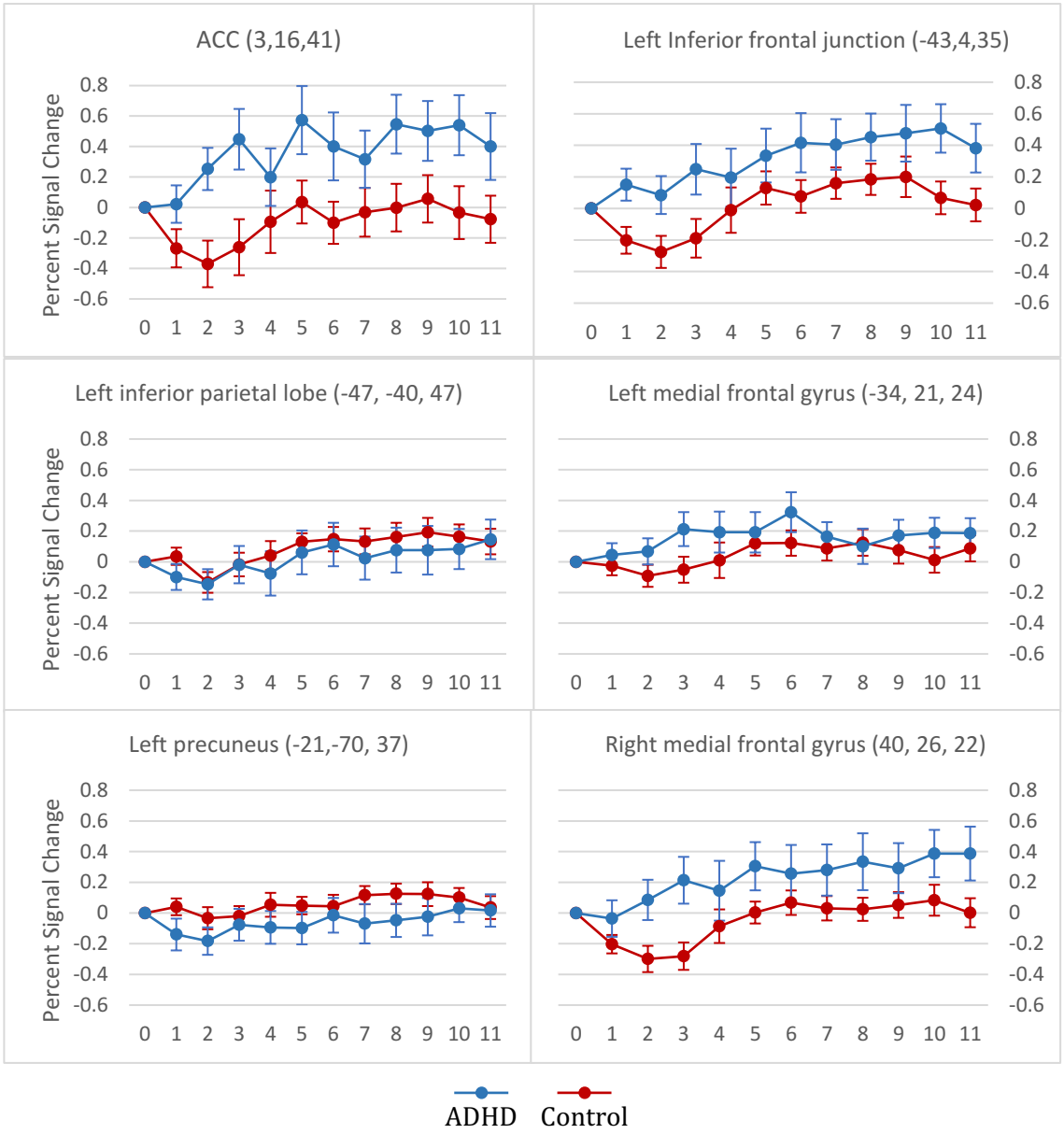


Figure 10-1: Difference between advanced incongruent and congruent activations as a function of scan number from stimulus onset (TR=3.0s), comparing control and ADHD participants at baseline by region of interest. Values below 0 indicate less activation in congruent than advanced incongruent stimulus conditions.

It can be seen from temporal evolution of the mean activation difference graphs (Figure 10.1) across the ROIs analysed that the most significant differences were detected in the ACC inferior frontal junction and the right medial frontal gyrus. The other two regions of interest did not have any differences detected at any of the 11 scan points ($p>0.05$). A summary of results is presented below in Table 10-2.

Table 10-2: Number of scan points with a significant difference (t-test) between ADHD and Control participants by region of interest.

	BA	x	y	z	Significant scan points ($p<0.05$)
Anterior cingulate cortex	24	3	16	41	5
Left inferior frontal junction	9	-43	4	35	4
Left inferior parietal lobe	40	-47	-40	47	0
Left medial frontal gyrus	13	-34	21	24	0
Left precuneus	7	-21	-70	37	0
Right medial frontal gyrus	46	40	26	22	2

The next part of the analysis path was to find the mean differences between ADHD and Control groups across the scans 1-11. These mean differences (with Standard errors) in percent signal change are shown as bar graphs in Figure 10-2.

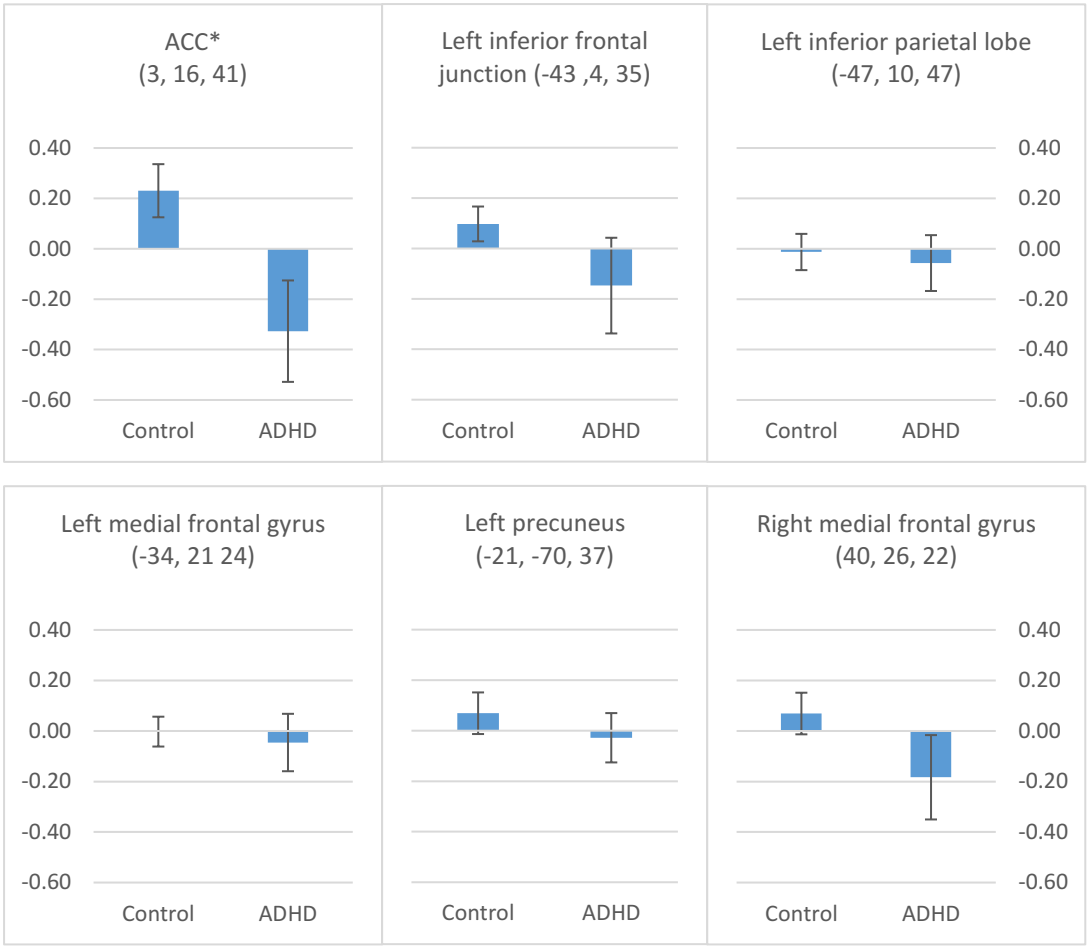


Figure 10-2: Average change in activation between T1 and T2 across supplementation groups for 6 regions of interest, measured in % signal change (*p<.05, **p<.01).

Invoking the subset of regions that show fMRI differences in a meta-analysis for the Stroop task, the Anterior Cingulate Cortex (ACC) and the Left Inferior Frontal Junction (LIFJ) (Laird et al., 2005), a repeated measures mixed ANOVA was carried out.

There were no significant differences between ROIs as could be intuited from Figure10.2 comparing the similar mean activations in ACC with LIFJ. However, the ADHD versus Control comparison was significant ($F_{1,44}=5.23$, $p=.027$). Post-hoc analysis shows that the effects were rather greater in the ACC compared with the LIFJ.

Table 10-3: Post-hoc difference in between group (ADHD vs Control) activation in the ACC and LIFJ.

	ACC	LIFJ
Mean Activation Difference	-0.56 %	-0.25 %
t-ratio	-2.61	-1.33
df	44	44
Prob>|t|	0.01	0.19

fMRI analysis of supplementation groups

In an approach similar to that used to find whether there were differences in performance between the ADHD and Control groups, the fMRI dataset was analysed with respect to the Formation given (Placebo, EPA, DHA).

As an initial stage, the time courses for difference graphs the activation across the ROIs for Advanced incongruent condition – Congruent condition were graphed (See Figure 10.3).

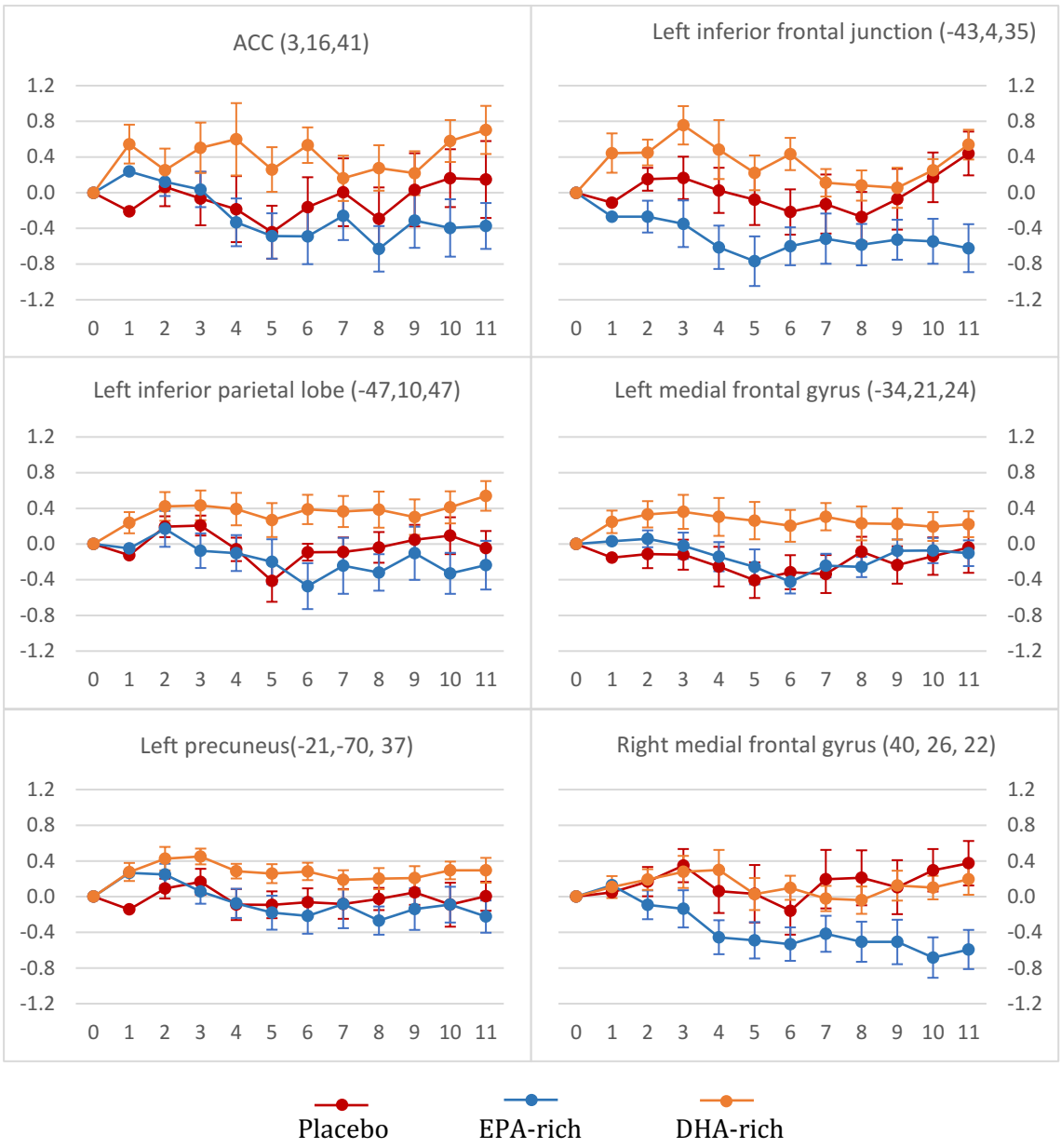


Figure 10-3: Scan time-courses of fMRI differences between Advanced Incongruent and Congruent task conditions, by supplementation group, for the 6 ROIs (measured in % signal change).

The general trend of data was established by evaluating the mean fMRI signal percent for each supplementation group (see Figure 10-4).

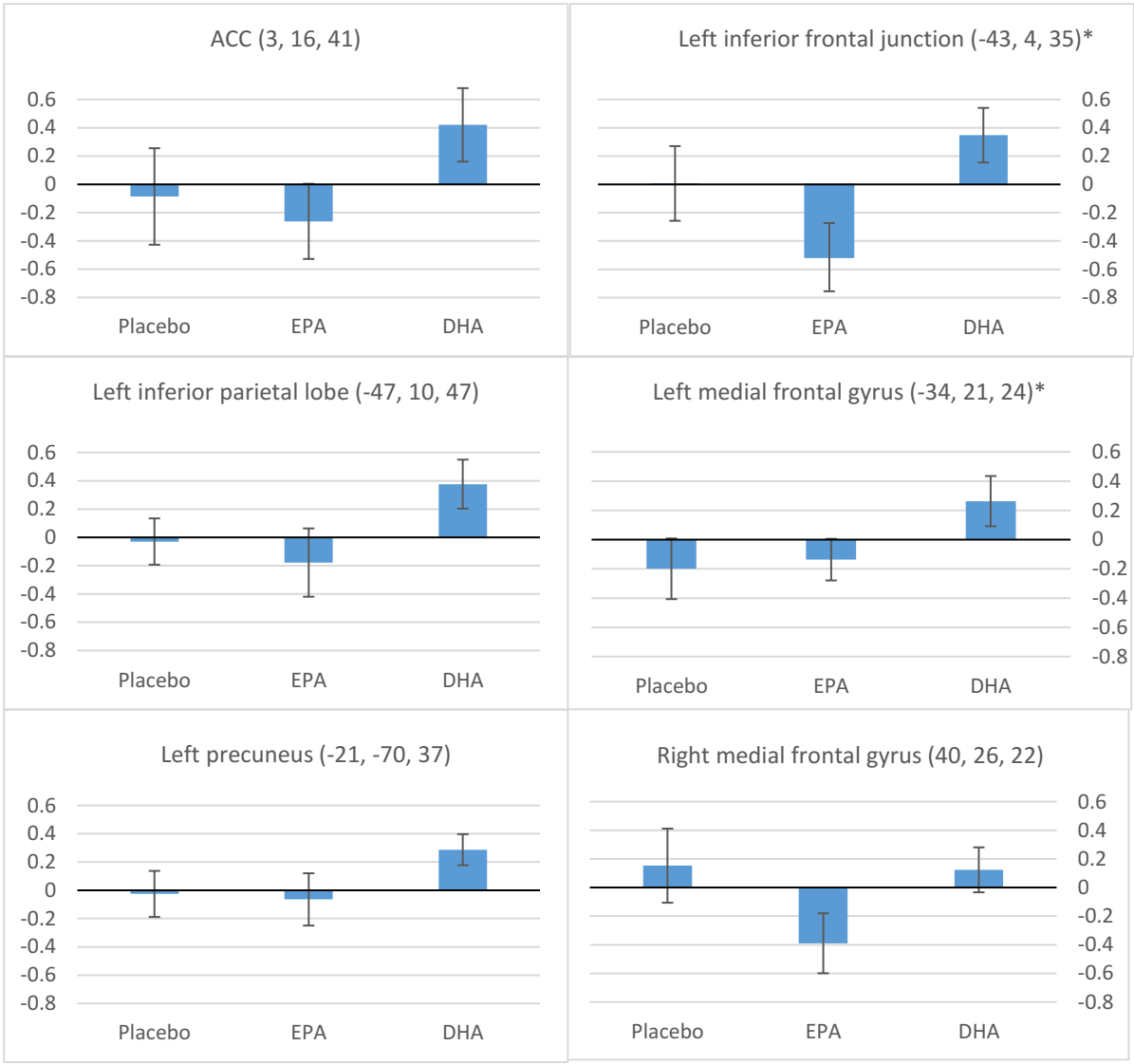


Figure 10-4: Average change in activation between T1 and T2 across supplementation groups for 6 regions of interest, measured in % signal change (*p<.05, **p<.01).

No significant overall differences were detected between the supplementation groups in the anterior cingulate cortex, left inferior parietal lobe, left precuneus and right medial frontal gyrus ($p>0.05$). Significant overall differences ($p<0.05$) were detected between the supplementation groups in the left inferior frontal junction and the left medial frontal gyrus.

Further exploratory analysis finds the regions identified in the meta-analysis (ACC and LIFJ) as showing some differences between supplementation. In order to analyse the differences in percent signal change between the supplementation groups, a one-way analysis of variance was conducted. This was completed on the average of all eleven non-zero difference points. Entering the ACC and LIFJ into an ANOVA with a between-group of formulation resulted in significant difference for the LIFJ, but not the ACC.

As the Formulation variable contains 3 different conditions, there are 3 two-way comparison that were tested. The contrasts investigated were comparisons of both EPA and DHA to placebo, and also to each other. This is displayed in the Table 10.4 below.

Table 10-4: Contrasts between supplementation groups by region of interest

	Overall significance	Contrast 1 (Placebo and DHA)	Contrast 2 (Placebo and EPA)	Contrast 3 (EPA and DHA)
Anterior cingulate cortex	>.05	>.05	>.05	>0.05
Left inferior frontal junction	.011*	>.05	>.05	0.00**
Left inferior parietal lobe	>.05	>.05	>.05	0.02*
Left medial frontal gyrus	.049*	.026*	>.05	0.04*
Left precuneus	>.05	>.05	>.05	>.05
Right medial frontal gyrus	>.05	>.05	0.040*	0.04*

*p<.05, **p<.01

10.10 Discussion

In this chapter, the effects of a 12 week EFA supplementation on functional brain imaging are reported. Participants were given one of either a placebo, EPA-rich or DHA-rich supplementation to take for a 12-week period. The three treatment groups were comparable in terms of intelligence measures and demographic variables. Similarly, there were also no differences between the ADHD and control participants in terms of these variables.

Functional brain activation- baseline

Based on previous research, it was predicted that there would be differences in activation between the control and ADHD participants, particularly in intersection of brain areas that are associated with primary symptoms of ADHD such as attention, and the task used to evoked brain activity such as the Stroop colour-word task. As predicted, the findings of the fMRI study showed significant activation differences between the ADHD and control participants in the ACC, an area purported to be associated with cognitive control and attention (Makris et al., 2010).

It was found that the activation patterns significantly differed between the ADHD participants and the control participants in the ACC as shown in Figure 10-1. The ADHD

participants had a higher percentage signal change comparing a difficult version of the Stroop task compared with the easy (Congruent) version where no interference was expected. In the absence of significant differences in behavioural results, this suggests that the ACC of the ADHD participants works harder to achieve the same results when under conditions of cognitive demand.

While there were 6 regions of interest that were potential sites of activity, the detailed statistical analysis was reserved for two of these – the ACC (anterior cingulate cortex) and the LIFJ (left inferior frontal junction), on the basis of common activation under Stroop interference (Laird et al., 2005). The ACC is of primary interest to this study due to its relationship with attentional control and memory, while the LIFJ has more recently been distinguished as playing a pivotal role (Brass, Derrfuss, Forstmann, & Cramon, 2005) in cognitive control and is both functionally as well as structurally distinguishable from the dorsolateral prefrontal cortex. The inferior frontal junction is located between the premotor and prefrontal cortex and is made up of Brodmann's areas 6, 9 and 44. A meta-analysis investigating frontal activations from colour-word Stroop studies found significant clusters of activation in the IFJ (Derrfuss, Brass, Neumann, & Von Cramon, 2005). This was supported by a second study by the same research group. Neumann, Lohmann, Derrfuss, and Von Cramon (2005) found the IFJ to have significant activation during the Stroop task. There were two major differences between the studies. Neumann et al. (2005) used replicator dynamics to identify dominant areas of activation whereas the Derrfuss et al. (2005) study used only frontal lobe co-ordinates and therefore detected less activation clusters. The validity of these results is strengthened by how the different methods yielded similar results. The research also provides a theoretical basis for the investigation of differences between Control and ADHD participants in the IFJ, because if the IFJ is associated with cognitive control, then it would be expected that ADHD participants would have differences in activation in this area. These results provide evidence for the role of the IFJ in the Stroop paradigm and cognitive control, supporting the findings of this research.

Functional brain activation- after supplementation

Based on previous research, it was also predicted that there would be differences in activation between the supplementation groups after a 12-week supplementation period. When looking at the effect of supplementation, the difference between the before and after supplementation scans was calculated. As predicted, the findings of the fMRI study showed significant activation differences between the placebo, EPA and DHA groups within the ROIs playing a strong functional role in cognitive and executive control.

While determining the exact biological mechanisms which underlie the changes in brain activation are difficult in human studies and beyond the scope of this study, the findings do support previous research suggesting differences in the neurophysiological effects of these two acids. There are many possible explanations for this including the speed at which EPA and DHA are incorporated into the cell membranes. Metherel, Armstrong, Patterson, and Stark (2009) found that EPA and DHA respond to dietary intake differently in blood. EPA levels, as a result of supplementation, have been found to change rapidly in blood, sometimes in a week or less (Di Stasi et al., 2004), whereas DHA levels change over a longer time period (Metherel et al., 2009). This could be partially due to EPA being taken up in the outer layer of the cell membranes whereas DHA is incorporated into inner cellular membranes. DHA has been found to take up to 12 weeks to be maximally incorporated into cellular membranes (Di Stasi et al., 2004). As the supplementation period of the study was only 12 weeks, it is possible that this may contribute to the results obtained.

Other explanations

While the concept of neural efficiency provided a central theoretical focus for this study, other explanations need to be sought. It is possible that the higher activation of the ADHD brains at baseline is a result of the anxiety, stress or the effort required to concentrate on the task as opposed to actual differences in the activation of the ACC when completing the cognitive task. This is plausible as people who suffer from ADHD also have a higher incidence of these symptoms. However, this higher activation in the ADHD may also be a reflection of the extra effort required from the ADHD brain to perform at about the same level as the Controls.

Methodological considerations and Limitations

There were issues with the study that need to be taken into account when drawing conclusions from this data. Due to a technical malfunction in the button press sensing system when collecting the data, the behavioural data of the fMRI tasks could not be collected and the behavioural data used was collected outside the magnet. The specific problem was that the button that was being pressed in the scanner was not being accurately recorded by the program. This means that the cognitive data that was compared with the fMRI data was collected outside of the scanner. It was decided not to use data if it was not believed to be valid or reliable, as this may lead to incorrect conclusions. As an alternative, cognitive data that was collected under experimental conditions outside the scanner was used. While this is not ideal, it does present some advantages. The data collection was consistent across all groups, so all of the groups were

equally affected. Additionally, the data being collected outside the scanner means that any anxiety or stress caused by the fMRI environment would be negated.

Another consideration is the number of participants involved. The power of the analyses may not have been strong enough to detect changes in activation as a result of the EFA supplementation. Additionally, while the parallel design of this experiment has its advantages such as a lower time commitment from participants and a tighter budget, it must be noted that there is variation in brain activation between individuals. If a crossover design with a washout period had been used, this variability would be minimised. However, this was not possible due to time and budget constraints of the study.

In conclusion, the fMRI results of this study suggest that patterns of neural activity while completing the Stroop task differed between ADHD participants and control participants at baseline. Additionally, the results also suggest that EPA-rich and DHA-rich supplementation have different effects over a 12-week period. This change in activation levels between EPA and DHA supplementation groups could be due to numerous biological mechanisms including but not limited to cerebral blood flow, inflammation or blood oxygenation, or differences in inflammatory effects of different oils. With further research funding, larger populations could be recruited, making more detailed statistical analyses of dietary supplementations on different clinical cohorts possible. Future research could investigate the presence of biomarkers in these areas to further determine the effect of EFAs on cognition.

Chapter 11 Integrative Analysis and General Discussion

11.1 Abstract

This thesis explored the effects of dietary supplementation with essential fatty acids on cognitive performance, cardiovascular function and brain activation in young adults, both with and without attention deficit hyperactivity disorder (ADHD). Such a process required the measure of essential fatty acid levels in the two participant groups as well as establishing whether there were differences at Baseline in terms of cognitive performance for tasks involving perception, attention and memory and in brain responses to such tasks. The purpose of this final chapter is to weave together the various threads of empirical discovery to come to a sophisticated view of the overall differences between ADHD and Control groups. The theoretical framework employed is the idea of "neural efficiency", as a means of relating brain activity and behavioural performance. The purpose of the general discussion is to tie together the results of cognitive testing, cardiovascular measures and functional magnetic resonance imaging to establish the degree of support for the theory of neural efficiency. It was found that Haier's original idea is overly simplistic, and ways to extend the theory are laid out. The findings of differential effects of essential fatty acids are discussed in terms of their potential for benefit that might arise from treating those with ADHD using EFA supplementation.

11.2 The Key Findings as Inputs to Theoretical Discussion

Baseline results

As expected, the experimental ADHD sample recruited on a history of clinical diagnosis showed significant differences when ADHD and Controls were tested on Conners self-report and observer scales. Surprisingly, at baseline, the levels of EFAs in the ADHD group were not significantly different from those of the Control group. This is despite previous literature suggesting lowered EFA levels as a characteristic of ADHD (see section 4.10).

The lack of difference could also be a result of increased omega-3 intake due to a popular belief of the therapeutic effects of EFAs on cardiovascular function as well as cognitive function. The influence of media and advertising may have played a role over recent years. There are a variety of omega-3 products on the market claiming many different health benefits, including cardiovascular health and brain function amongst others, and there is a strong unambiguous advertising campaign behind these products. This is evidenced by supplements containing omega-3 representing 20% of the market share of nutritional supplements, second only to multivitamins (Themedica, 2009). Either consciously or

unconsciously, this may have led to an increased intake of omega-3 fatty acids through dietary intake both in ADHD and control participants.

Comparisons between diets in the international literature must be undertaken with a degree of caution to avoid making inaccurate generalisations. The benchmark values of the literature review of the current thesis were drawn from previous research which includes contemporary research in combination with estimated values from the past, as well as drawing from different countries with different socioeconomic situations and policies, see section 3.3 of this thesis.

11.3 Supplementation Results

Control versus ADHD

Over a 3-month period, participants were supplemented with either EPA-rich, DHA-rich or placebo supplementation.

Bloods

Analysis of bloods after the supplementation period showed the effects of the different supplements. There were no differences between control and ADHD participants in EFA levels after supplementation, either at an absolute or relative level. However, there was a satisfying increase in the target EFA found when analysed by supplementation groups (as shown in Figure 7.5), with EPA increasing in the EPA rich diet, DHA increasing for the DHA rich diet and the placebo diet causing no significant change in the two target omega-3 EFAs.

Cardiovascular

As shown in Chapter 9, there were few cardiovascular differences detected across the 12-week supplementation period for either control or ADHD participants. While mean common carotid blood flow was greater for the ADHD group compared with controls after supplementation, the marginally significant t-test result did not survive adjustment for multiple comparisons. The absence of cardiovascular differences between ADHD and Controls was true for both absolute values after supplementation values and for relative differences between the time points. It must also be noted that the participants in this trial are all relatively young (below 40), where cardiovascular abnormalities are rare.

In terms of comparison of supplementation group, significant differences were found, mainly in terms of blood pressure and blood velocity. EPA (and Placebo) produced a significant reduction for each of 4 measures of blood pressure (central and peripheral, systolic and diastolic pressures). DHA supplementation, by comparison, had no effect on any of the blood pressure measures. The same split between supplementation groups

occurred for Common Carotid Blood Flow Velocity, where Placebo and EPA groups showed no effects of supplementation, while the DHA showed a reduction in velocity after supplementation. These findings point to a realisation that not all omega-3 oils are the same in effect, a realisation that must be considered when other effects, such as brain imaging, are compared.

Cognition

Contrary to hypotheses, there were few differences detected between control and ADHD participants in the cognitive outcomes measured by this trial. Overall, there were no differences between control and ADHD participants after supplementation. However, some differences were detected between the Control and ADHD participants on relative performance between the two time points for a subset of tasks. Simple recognition memory reaction time decreased in controls but not the ADHD group. As the original hypothesis was based on the ADHD participants having lower EFA levels than the control participants, the null overall result in cognition could simply relate to the blood results in section 6.6. However, the explanation may be more complex, as investigated later in this chapter. Previous research has suggested that the SUCCAB task most sensitive to cognitive change is the spatial working memory task (Pipingas et al., 2008), but this was not the case with the current supplementation study. However, while a particular cognitive task may exhibit less noise than others and hence be likely to show sensitivity, also one has to take account of the nature of the supplementations used. In (Pipingas et al., 2008) a pine bark supplement with strong antioxidant properties was employed. There should be no expectation that antioxidants in general should show that same cognitive (or brain) effects as EFAs. In the current study some differences in delayed recognition memory were detected. While the previous research focussed on age-related cognitive differences, this study focused on the difference between ADHD and control participants. ADHD participants may be able to recall items within a short period whereas perhaps they have more trouble when there is a longer recall period.

In terms of differences related to supplementation, the findings of chapter 8 are quite clear. EPA and DHA were found to have a different pattern of effects on psychophysiologically measured cognitive processes. For some tasks (eg Simple Recognition Memory), all supplementations reduced reaction times. While this might be interpreted as a consequence of practice effects, a 12 week gap is rather longer than one would expect such effects to last.

The cognitive testing (performance and reaction time) also gives clear evidence that the three versions of the Stroop task: Stroop Congruent, Stroop Incongruent, and Stroop Advanced Incongruent are ordered in terms of cognitive load corresponding with each level of increased difficulty.

Functional Brain Activation-fMRI

It was hypothesised that differences in activation would be detected between the control and ADHD participants, particularly in brain areas that are associated with primary symptoms of ADHD such as attention. As predicted, the data from the fMRI study showed significant activation differences between these groups in the anterior cingulate cortex, an area associated with cognitive control and attention (see section 10.3). The higher percentage signal change detected in the ADHD participants when compared to the controls indicates more activation in this area. Indeed, qualitatively, the task-related BOLD signal change remains raised for over 30 secs in the ADHD group relative to the Controls (see Figure 10-1).

11.4 The Neural Efficiency Theory

Smart brains work less hard. Such a simple idea was put forward by Richard Haier in the 1980s in order to explain the first data comparing brain metabolic activity and cognitive performance. As discussed earlier in section 10.2, the theory of neural efficiency was developed by Haier (Haier, Siegel, Tang, Abel, & Buchsbaum, 1992; Haier et al., 1988), and it suggests that differences in human cognitive ability is not simply a function of how hard the brain is working, but rather the efficiency of the brain processes that are being undertaken. This means that individuals with a higher IQ may require less neural resources when undertaking higher order cognitive processes than that of individual with a lower IQ (Haier, Siegel, et al., 1992; Haier et al., 1988).

Neural Efficiency as a Between-subjects idea

While this was found at the time of the research, this was almost 30 years ago and technology has come a long way since then. When this theory was developed, fMRI technology was in very early developmental stages – indeed the original studies were performed on glucose utilization using PET scans. Since their 1988 research, Haier and colleagues have built on their original research and published numerous research papers investigating the correlates of intelligence through the use of fMRI, PET and EEG (Haier, Siegel, et al., 1992; Haier, Siegel Jr, et al., 1992; Larson, Haier, LaCasse, & Hazen, 1995). This research provided further evidence suggesting that the brains of participants with lower IQ scores need to work harder than the brains of participants with higher IQ to

achieve comparable levels of cognitive performance. While this did demonstrate the theory of neural efficiency, it must be noted that this was demonstrated using IQ data versus cognitive performance and care must be taken when using these comparisons to make general statements.

A further illustration of the theory of neural efficiency was provided by Kwon and colleagues in 2001 (Kwon et al., 2001) using a 1-back and 2-back working memory task conducted on Fragile X females versus Control females aged between 10 and 23. Fragile X syndrome (FX) is a genetic condition which is characterised by intellectual disability, behavioural difficulties and learning difficulties. In the fairly easy 1-back condition, fMRI analysis showed the FX group had greater BOLD activation than a comparison group of typically developing young women and performed above chance but significantly worse on the task. This conforms with the idea of neural efficiency. However, when the visuo spatial location task increased in difficulty to a 2-back working memory task, the pattern of activation changed. The FX group showed mixed results with an increase in some regions and a decrease in others with the increase in activation being relatively smaller than the control group. In terms of performance, both groups performed at a lower level in the more difficult 2-back task, but this was more pronounced in the FX group. In the inferior frontal gyrus region BOLD activation was reduced for the FX group for the 2-back compared with the 1-back task, while the Control group showed the reverse functional changes, increasing activation level while maintaining performance. This could be interpreted as the FX group not being able (either consciously or unconsciously) to upregulate their performance with increasing difficulty.

Neural Efficiency for within-subject analysis of the effects of supplementation.

Since the research outlined above, the idea of neural efficiency has been expanded to explore within-subject effects of supplementation by essential fatty acids. Using multi-focal visual evoked potentials (VEP), Bauer et al. (2011) predicted that VEP nonlinearities would be very sensitive to neuronal recovery after firing. The explanation for this notion comes from the idea of a temporally linear system as one that can respond in exactly the same fashion when stimulated at high frequency as at low. The measure of an inability to respond in such a way at high frequencies is to populate higher order kernels in the Wiener kernel expansion. Thus second order amplitudes give a measure of the efficiency of a neural population (generating the VEP). Using a double blinded crossover design with a 30-day supplementation period, this research found that EPA-rich supplementation reduced the magnocellularly generated non-linearity over occipital cortex to a greater

extent than a DHA-rich supplementation. This pattern of reduced non-linearities suggest a quicker recovery in this group, indicative of a higher level of neural efficiency.

In another arm of the same cross-over study involved fMRI BOLD activation measurement (Bauer et al., 2014), the authors concluded that EPA-rich supplementation improved neural efficiency and cognitive performance in a typically developing young adult population. This was demonstrated by a reduction in functional brain activation in the left anterior cingulate cortex along with an improvement in cognitive performance. This pattern of results was not observed following DHA-rich supplementation.

However, interpretation of results of different experimental protocols requires close inspection of the method used to measure cognitive performance. The time between brain scans (TR) is generally two to three seconds. Hence, if there is one trial per TR (repeat time), it is possible that some people may solve the tasks within the TR. They may have less activation within the TR because they finished the task quicker, rather than having less activation while completing the task. If the tasks are presumed to be delivered at a consistent time point, then a possible interpretation of the negative correlation between functional activation and IQ might be simply due to the proportion of time that the brain is actively working on the problem.

Why is the Neural Efficiency Theory too simple?

fMRI BOLD data is most often presented as a comparison between task and baseline (or resting) performance, or between one group and another on similar measures. Across the brain, typically two-tailed effects are observable. Some parts of the brain (e.g. the pre-cuneus) are more active when a participant is not performing a task – hence the resultant BOLD would show a negative activation in a comparison of task-rest activation. How then can the simple sign of higher neural efficiency - lesser brain activity for a particular degree of performance be measured if there are both positive and negative measures across the brain?

Thus, while neural efficiency theory works well for a one-tailed comparison, it does not work for a two-tailed comparison. Negative coefficients occur as well as positive ones, as an attentional network can work both ways. When describing attention William James (1890) proposed that “Focalisation, concentration, of consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others”.

How can the Neural Efficiency Theory move forward?

One way forward with the neural efficiency hypothesis is to adapt it to work with two-tailed hypotheses. Using independent components analysis on the activations patterns,

networks can be identified and associated with the strength of the signals. Theoretically such networks can have contributions with either positive or negative sign. The changes observed could then be coded in terms of the changes in strengths of the various components and such strengths could be arranged to be positive definite in nature. Future research could examine the strength of such networks forming a model in which neural efficiency could be quantified.

In order to investigate further, an understanding needs to be reached in terms of what neural efficiency means to brain activity. Current brain imaging methods do not typically assess maximum rate of working, but rather look at BOLD activation for a standard, fixed rate of stimulus presentation. Thus, the current results are partially determined by task design as they are conducted in the fMRI. If a person completes tasks that were aimed to maximise brain activation then stimulus presentation should not be coupled with a standard time, but simply follow the previous response. If so, peak reaction times and performance would be measured along with peak activation.

11.5 Does the Neural Efficiency Theory help to tie together all the findings?

Performance on cognitive tasks depends on our cognitive and perceptual set. Even if overt feedback is not given on a trial by trial basis, each participant has their own performance 'set" where they will apply more effort if they feel that they are starting to perform worse over the duration of a task. This immediately suggests individual differences in this attentional set. More importantly, given the nature of the symptoms of ADHD including difficulty in maintaining attention, increased impulsivity and hyperactivity, such individual differences are probably reflected in a group-wise fashion between the ADHD and Control populations. Yet as shown in section 6.6 (p95) performance at Baseline was not dramatically different between ADHD and Control groups. This is where it becomes important to consider how the brain responds to cognitive demand and to conscious recognition of impaired performance. If awareness of a lack in performance causes compensatory up-regulation of brain activity in a systematic fashion, then such deficits might be masked when only behavioural data is compared.

Neural efficiency theory, as presented in this thesis, provides a potential way of looking at the relationship between the different variables explored in this thesis. As discussed earlier in section 10.2, the theory of neural efficiency suggests that individuals with a higher IQ may require less neural resources when undertaking higher order cognitive processes than that of individual with a lower IQ (Haier et al., 1992; Haier et al., 1988). This theory suggests that differences in human cognitive ability are not necessarily a

function of its absolute capabilities, but rather the efficiency of the brain processes that are being undertaken (Haier et al., 1988).

Thus in the simplest fashion, the combination of similar perceptual performance (ADHD *cf.* Control) at Baseline, shown in section 6.6 (p95),coupled with generally raised levels of BOLD activation as shown in Figure 1 of Chapter 10, is consistent with the notion that those with ADHD had to work harder in terms of brain activity in order to reach cognitive performance of approximately the same level as the Control participants. However, the difference in activation is not uniform across all brain regions – it is well accepted that different tasks activate subsets of brain regions to different amounts, depending on task and individual. Following on with this logic, one would expect certain regions of the brain to be informative about neural efficiency, while others may give no (or little) information. Hence, activity in the Anterior Cingulate and Inferior Frontal Junction ROIs are strongly informative, while activity in the pre-cuneus and Left inferior parietal lobe give little information. While this discussion clearly identifies how the Neural Efficiency hypothesis is perhaps too simplistic for complex data types (fMRI, EEG, MEG), it is just as clear that its revision will require considerable novel effort with innovative research design

Evaluation of the Neural Efficiency framework in its between-subject version should therefore depend on correlational activity between behaviour and brain activity, and particularly comparing across groups. Such comparisons in this thesis are restricted somewhat by compliance – particularly in terms of participant drop-out at the second measuring time. However, as an illustration, the process is considered, looking at a comparison of such correlations for the Control and ADHD groups. Figure 11.1 shows the effect of cognitive demand, by differencing the reaction times (adjusted by performance) between the Stroop task conditions of increasing difficulty.

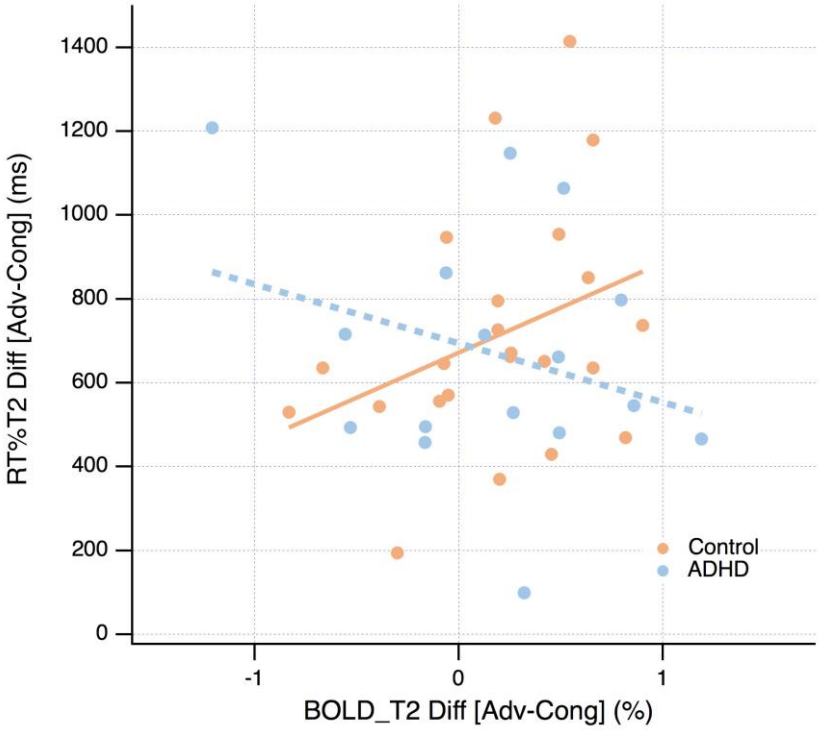


Figure 11-1: Correlation between the differences for the Advanced-Congruent Stroop task (RT moderated by performance) of Chapter 8 plotted against BOLD activation difference for the same task comparison (from Chapter 10)– comparing ADHD versus Control participants.

The data, taken from testing time T2, looks at the difference in reaction time dived by performance for the Stroop Advanced compared with the Stroop Congruent tasks. Different regression lines were found for the ADHD compared with Controls, though the differences were only marginally different (Pearson correlations: Control 0.342; ADHD -0. Difference in correlation estimated via permutation testing (LabView) against the null hypothesis that the correlation data was from the same population. Ranking the empirical difference in correlation against the 1000 permutations calculated yielded p=0.082, two-tailed).

The same approach was used with the analysis of Supplementations.

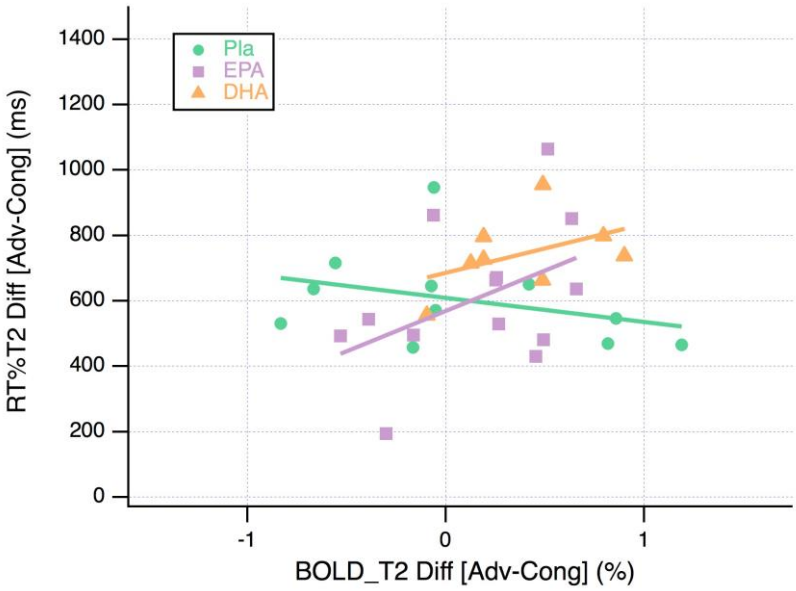


Figure 11-2: Correlation between the differences for the Advanced-Congruent Stroop task (RT moderated by performance) of Chapter 8 plotted against BOLD activation difference for the same task comparison (from Chapter 10) comparing supplementation groups.

Given the small numbers in each group, the permutation testing for correlation difference was applied to Placebo versus a combination of the EPA and DHA data (Correlations: Placebo -0.342; EPA + DHA 0.493. Difference in correlation estimated via permutation testing against the null hypothesis that the correlation data was from the same population (ie that supplementation type made no difference). Ranking the empirical difference in correlation against the 1000 permutations calculated yielded p=0.013, two-tailed).

Thus, despite the reduced sample size by the end of the study, there is clear evidence that the consumption of omega-3 EFAs altered brain-behaviour relations (here the relation between response time/performance against brain activation).

While the theory of neural efficiency has been invoked to explain some of the findings of this thesis, it cannot fully explain all of the data and its weaknesses must be considered. Inhibition and excitation of the brain are complex processes and it has been well established for over a century that the attentional processes leading to improved performance involve suppression as well as activation.

It is also plausible future models of neural efficiency will have to take into account other behavioural factors. For example, the higher activation of the ADHD brains at baseline may receive a contribution from different levels of the anxiety, stress or the effort required to concentrate on the task. Such factors may contribute in a complex fashion to actual differences in the activation of the ACC when completing the cognitive task. This is

plausible as people who suffer from ADHD also have a higher incidence of these symptoms. However, this higher activation in the ADHD may be a reflection of the extra effort required from the ADHD brain to achieve the same result.

11.6 Contribution

While there is considerable behavioural research on the effects of certain EFAs on humans, there are gaps in the literature that this thesis fills. In particular, there is relatively little literature reporting the effects of EFAs on cognitive function and brain function in young healthy adults, with the majority of research focussing on clinical or ageing populations. In a similar fashion, there is an abundance of research, largely behavioural, conducted on people suffering from ADHD, but this research is almost exclusively conducted on children and adolescents.

11.7 Thesis limitations and future directions

Despite the researcher's best intentions of minimising the limitations of this study, there were some limitations of the methodology used in this thesis and these must be taken into account when examining the findings and taken into account for future research. These limitations will be discussed in the following section.

Participant limitations

Recruitment of participants with a diagnosis of ADHD proved to be more difficult than anticipated. This was due to several reasons. While some studies are able to employ full time staff and dedicate months for recruitment, the resources of this trial did not facilitate this. Additionally, by nature ADHD participants have issues with attention and some lacked the time management and organisational skills required to partake in this trial. While the researchers made every effort to overcome these issues, sometimes this proved to be a barrier to participation, and particularly in a repeat visit design as was used here

One limitation of the thesis is in regards to clinical participants and refers to the presence of a clinical diagnosis. The researchers of this study endeavoured to collect a diagnostic report from each participant, written by their clinician confirming the diagnosis of ADHD. At the beginning of the study, the researchers attempted to only recruit adults with a clinical diagnosis of ADHD. However, due to logistical problems in some cases, this report was difficult to obtain and therefore the researchers could not confirm the diagnosis of some participants in this study. Researchers asked every ADHD participant for a copy of their diagnostic report, but possibly due to the nature of the condition, not every participant was compliant with this request. This request was followed up by emails and telephone calls, but after numerous attempts the researchers still accepted the

participants into the study. Never-the-less, the difference between ADHD and Control groups on the Conners ADHD self-report and observer scales lend support to manifest behavioural difference between groups

An additional issue with the thesis was that the ADHD participants were not all receiving the same pharmacological treatments. Some of them were on stimulant medication, and some of them were not on any medication. Initially participants were sought who were not medicated in order to keep the participants as similar as possible, but this proved to cause many recruitment issues. While this is not ideal, the participants who were on medication had not changed their prescription in the month prior to the study, and their medication levels remained stable throughout the supplementation period. Additionally, participants who were medication naïve at the first testing session remained this way for the duration of the study. Further research could aim to replicate the findings of this thesis in both medicated and medication naïve samples. This would strengthen the findings of the thesis and suggest real world implications.

One possible issue with the sample is the issue of gender bias. The ADHD sample had a sample of 50% female and 50% male, and the control sample was 66% male and 33% female. This occurred because previous literature showed that ADHD occurs more predominantly in males than in females and is characterised by a gender ratio of approximately 3:1(Ramtekkar, Reiersen, Todorov, & Todd, 2010) (American Psychiatric Association, 2000b). As controls were recruited first, in anticipation of there being more males than females in the ADHD group, a ratio of 2:1 males was recruited. The ADHD sample that was recruited did not represent that finding, as equal numbers of both genders were recruited. As noted previously in section 4.6 of this thesis, many claims of gender bias in ADHD come from paediatric sample populations. (Biederman et al., 2002; Gershon, 2002)Future studies could attempt to address this issue by recruiting simultaneously and adjusting ratios as recruitment progressed. In addition to this, the age of the sample population should be taken into account when determining the gender ratio of the study.

Assumptions of the BOLD technique

While much research has been conducted using fMRI, the assumptions of the method must be considered when drawing conclusions from the data. The concept of fMRI is based on the blood oxygen level dependency (BOLD) response. This technique assumes brain activation is coupled with changes in blood flow to the area of the brain, with more blood flow in certain areas indicating higher levels of neuronal activity. fMRI can be seen as an indirect measure of neural activity, as it measures the magnetic response to the

BOLD response rather than the activity itself (Arthurs & Boniface, 2002). The accurate interpretation of this signal is dependent on understanding the underlying neural activity that creates the signal (Arthurs & Boniface, 2002) and this must be taken into account when interpreting the results.

Neural efficiency limitations

Another limitation of this thesis is that the explanation of neural efficiency for the findings of this thesis does not provide a full explanation of the data obtained. It must be noted that the issues outlined above with neural efficiency do not render the findings of this thesis wrong, but that caution needs to be taken when interpreting the results. The neural efficiency explanation for the data in this thesis is still manifestly good, but could be seen as too simplistic. This highlights the need for further research in this area to broaden the scope of the neural efficiency theory and expand the capabilities it has to explain neural phenomena.

It is outside the scope of this thesis to fully investigate all of the factors that could possibly have an effect on the variables investigated in this thesis. Other considerations that could be investigated include genetic factors (De Geus, Wright, Martin, & Boomsma, 2001), exercise (Cotman & Berchtold, 2002), and mental health amongst other factors. It is important to recognise the potential influence of these variables, as they add another level of complexity of the research into this area.

The data collected from this thesis suggests that more research is required in the area of neural efficiency. While the theory of neural efficiency cannot yet handle deviations in the data and essentially works in a unidirectional manner, future research could address this issue. By furthering research into this are and examining the data in a different way, a more robust model could be developed to extend knowledge in this area.

Another future research direction could be the relationship between cognition and the cardiovascular system, one of the many mechanisms that have been suggested for the relationship between essential fatty acids and cognition (Kandel et al., 2000; Zlokovic, 2008). Relationships have been detected between cardiovascular performance and cognition through arterial stiffness, with certain cognitive domains such as working memory declining with elevated arterial stiffness(Waldstein et al., 2008). However, this was contradicted by the findings of a prospective population-based study which did not identify arterial stiffness as an independent risk factor for cognitive decline (Poels et al., 2007). These opposing research findings used different methodologies, and this may be what led to contradictory results. This thesis investigated the effects of EFA

supplementation on both variables individually, but future research could investigate the interaction between these variables in a more thorough manner. Future research in this area could help to clarify relationships between specific cognitive domains and arterial stiffness.

11.8 General conclusions

The general aim of this thesis was to investigate the behavioural, cognitive, cardiovascular and neural effects of EFA supplementation on control and ADHD participants. This thesis aimed to combine these aspects and investigate them using a neural efficiency theory for explanation.

In conclusion, this finding of this thesis suggest that omega-3 alters some aspects of neurocognitive performance in young adults. While no overall cognitive differences were detected between groups after supplementation, there was a pattern of differences between the two time observation points, with EPA-rich supplementation leading to more improvements than DHA-rich supplementation. This thesis provides further evidence for the theory of neural efficiency, demonstrated through the differences in neural activity while completing the Stroop task between ADHD participants and control participants.

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Appendices

Appendix i: Consent form for the omega-3 supplementation study.

Participant Information **Date: / /**

Full Project Title: Effects of fish oil on brain efficiency in a healthy adult population and in a population of adults presenting with Attention Deficit Hyperactivity Disorder (ADHD) symptoms.

Principal Researcher: Prof. David Crewther (Swinburne University of Technology)

Associate Researcher(s):

Dr. Andrew Pipingas (Swinburne University)

Prof. Sheila Crewther (La Trobe University)

Ms Isabelle Bauer (Swinburne University)

Ms Laura Sellick (Swinburne University)

This Participant Information and Consent Form is 9 pages long. Please make sure you have all pages.

Short Glossary to help you understand this document

ADHD = Attention Deficit Hyperactivity Disorder

BOLD = Blood oxygenation level dependent

BSI = Brain Sciences Institute

DHA = Docosahexaenoic omega 3 fatty acid

DTI = Diffusion Tensor Imaging

EPA = Eicosapentaenoic omega 3 fatty acid

fMRI = functional magnetic resonance imaging

TGA = Therapeutic Good Administration

VEP = Visual Evoked Potential

1. Your Consent

You are invited to take part in this research project. This Participant Information contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part in it. Please read this Participant Information carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this. Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project. You will be given a copy of the Participant Information and Consent Form to keep as a record.

2. Purpose and Background

Consumption of "good" and "bad" fatty acids is rising rapidly in Australian diets, however relatively little research has been carried out on the cognitive and brain function effects of consuming such diets. We are now used to the idea that diet is a critical factor in cardiovascular disease, with low docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) fatty acid diets. Mammals, including humans, are entirely dependent on dietary sources of omega-3 fatty acids to maintain proper tissue levels, whether for heart or for brain and cognition. Within the health food market, a number of claims have been made as to the effects of fish oil, such as improvements in cardiovascular health, and reduction of symptoms associated with behavioural disorders such as Attention Deficit Hyperactive Disorder (ADHD).

In some cases, ADHD profoundly affects a person's school, work and social life. Some

researchers claim that ADHD is due to a deficiency in omega-3 fatty acids however only a few studies have shown improved cognitive performance following fish oil supplementation. These studies were conducted in a population of young children and cannot therefore be generalized to a population of young adults suffering with ADHD.

Fish oils are also being marketed to parents with claims that they will make their children smarter, however little research has been conducted examining the basis of these claims. So the first aim of this study is to answer the question “do fish oils make us smarter?” Secondly, fish oil tablets are made of both EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) compounds. While the brain is made of largely DHA compounds the recent literature indicates that a higher EPA content may be more beneficial for cognitive health – improvement of memory, attentional and more demanding thinking processes. There are no studies known to the researchers involved in this study that have investigated cognitive neurological effects associated with consumption of different ratios of EPA to DHA. Thirdly, we are interested in evaluating cardiovascular and cognitive effects of EPA and DHA in a population of young adults presenting with ADHD.

Are you eligible to participate in the study?

To participate in the study you will need to be:

- 18-40 years of age
- Diagnosis of ADHD confirmed by a clinician (doctor/psychologist) or suspected ADHD symptoms
- No history of neurological disease or diabetes
- Not currently taking fish oil supplements
- Generally in good health
- No history of epilepsy
- No medication for cardiovascular or blood disorder
- You are required to attend 2 weekday daytime testing sessions at Swinburne University of Technology and have two blood tests over a three-month period.
- You may be randomly selected and invited to take part in 2 sessions of functional magnetic resonance imaging (fMRI) at the Austin Hospital.
- You will also have the option of continuing the supplementation for a further 3 months, with one extra testing session. If you choose to participate in this extension, you will be further compensated for your time.

3. Procedures

The study will be conducted during 2010/2011. You will attend two sessions at the Brain Sciences Institute (BSI), Swinburne University Hawthorn and at the Brain Research Institute at the Austin Hospital (if selected for the fMRI group), and will undergo blood testing.

We are testing the effects of taking 3 different fish oil supplements. You will receive either Diet A, Diet B or Diet C.

By comparing test results following each fish oil diet with initial testing conducted prior to the commencement of supplementation we will be able to investigate whether there are changes in cognitive functioning, in blood measures and cardiovascular function associated with supplementation with fish oil.

By comparing test results following each fish oil diet we will be able to investigate differential diet effects. That is, whether one fish oil diet is better than the other.

The testing that we will conduct is as follows:

Questionnaires

The first time you attend the Brain Sciences Institute we will ask you to read this form and if you agree, sign the informed consent form. This will mean that you are enrolled into the study. As well as taking your personal and contact details, we will ask you to complete a number of questionnaires asking general questions about your health, mood, dietary habits, behavioural traits and other demographic information. We may also ask you to provide an official report regarding your ADHD diagnosis. When you return to the BSI we will ask you to bring back any remaining fish oil supplements and your compliance sheet (check sheet to make sure that you are taking your supplements). Once you have had your blood test then you can commence taking supplements.

Computerized psychological tests

A number of computer-based psychological tasks will be presented to you. These tasks have been designed to test different aspects of thinking processes such as attention and memory. You will use a hand-held button box to make responses to stimuli presented on a computer screen. Detailed instructions will be given to you prior to performing these tasks. You will also be given adequate practice. A continuous performance computer task and an ocular pursuit that monitors eye movement will be used as objective measures of your abilities to concentrate for a longer period of time and the ability to ignore external distractions.

This procedure will last for about half an hour.

Recording of brain electrical activity

A visual Evoked Potential (VEP) test is similar to an electrocardiogram (ECG). The difference is that the recording discs are on the scalp to measure brain function, instead of on the chest to measure heart function. A small quantity of a water-based conductive gel will be used to make electrical contact between the scalp surface and the recording discs. This will be washed-off once data collection has been completed. The application of recording discs does not involve any breaking or piercing of the skin. A pattern on a computer monitor will change while you concentrate on the centre of the pattern. You will not experience anything unpleasant, and the procedure is completely pain-free. This technique will determine the speed of conduction of electrical responses through the brain and reflects the integrity/efficiency of neural tissues in response to visual stimuli.

This procedure will last for about half an hour.

Blood testing

Blood testing will be conducted on the day of your first testing session at the BSI and when you return after taking the assigned diet. A qualified nurse from the Brain Sciences Institute or the PhD students involved in this study under supervision of the qualified nurse will collect one or two drops of your blood by puncturing your fingertip with a sterile, disposable, lancing device. Participants will be instructed to fast for at least 5 hours prior to undergoing this blood test. Blood samples will be collected in a filter paper and stored in a secure -80 degrees Celsius freezer at the BSI Building on 475 Burwood Road in Hawthorn. All blood samples will be de-identified through coding, and your name will not be associated with your blood sample from this point onwards. Only the researchers at the BSI involved in this study will have access to the information which could potentially identify your data. Blood samples will be transported through a courier (FedEx) to the Omegametrix Blood Laboratory in Martinsried (Germany) where fatty acid analyses will take place. After the essential fatty acid analyses are completed, all samples will be destroyed and disposed of by Omegametrix. At a later time these blood measures will be compared with computerized psychological tests to investigate relationships between fatty acid levels

and brain function. Participants will be provided with a light breakfast or snacks prior to the commencement of cognitive testing.

Blood Pressure

Blood pressure and arterial stiffness will be measured to investigate whether fatty acids have an effect on the cardiovascular system. Blood Pressure will be measured using a self-inflating cuff device with a digital display. Arterial stiffness will be calculated using a non-invasive technique where a small pressure transducer is applied on the wrist over the radial artery. Pressure waveforms associated with your heart beat will be recorded and a computer algorithm will be used to calculate arterial stiffness.

Transcranial Doppler System

The effect of fish oil supplementation on cerebral blood flow velocity will be assessed using the Compumedics DWL Transcranial Doppler system. This non-invasive system measures blood velocity of the middle cerebral artery from a point near your ear using a handheld probe device the size of pencil. This device uses high frequency sound waves to measure blood flow velocity. This measurement will allow for the assessment of brain related changes due to fish oil status in the blood before taking fish oils and following supplementation with fish oils.

Neuroimaging techniques

You may undergo a Functional Magnetic Resonance Imaging (fMRI) investigation at the Brain Research Institute, Austin Hospital. combined with Diffusion Tensor Imaging (DTI). Participants will be asked in order of recruitment if they would like to participate. Because this methodology is expensive we do not have sufficient funds to test everyone. fMRI assesses the distribution of oxygenated and deoxygenated blood in the brain as a function of mental activity. With this test we will be able to investigate if fish oil diets have an effect on brain activity measured through the BOLD (Blood Oxygen Level Dependant) effect. DTI tractography is a technique commonly used in combination with fMRI to observe changes in the white matter. If fish oil diets do improve memory and other cognitive processes, then with the addition of fMRI testing we may be able to say if improvements are due to particular brain systems by comparing brain activity changes, blood flow variations and cognitive changes. While MRI scanning is associated with certain risks, these risks are addressed by way of a

safety screening checklist and consent form prior to MRI scanning. These documents provide more specific information about MRI scanning and check that you don't have any metallic implants, pacemaker, body jewellery, etc. that would prevent you from having an MRI scan. Also, some people may feel claustrophobic during MRI scanning. This is minimized by procedures that will be explained to you by the MRI technologist at the Austin Hospital prior to scanning.

Follow-up study

You will be asked if you wish to continue the fish oil supplementation for a further three months. This will involve one additional blood test and one additional behavioral and electrophysiological testing session at the BSI at the end of the six-moth supplementation period. If you have agreed to undergo an fMRI investigation in the first two testing sessions you may be asked to undergo a third investigation at the end of the six-month supplementation period. However, this will depend on the number of participants who agree to extend the supplementation period. This will take place on a purely voluntary basis, and is not part of the inclusion criteria for the 3 month supplementation study. If you do choose to continue with this supplementation, you will be compensated with an extra $50.

4. Possible Benefits

We cannot guarantee or promise that you will receive any benefits from this project. However, your research results will contribute to the research effort to help understand whether fish oil diets are beneficial and if EPA rich diets are better than DHA rich diets.

A summary report of the findings of the study will be provided to each participant within 3 months of the conclusion of the study.

5. Possible Risks

Fish oil supplements that we are using are Therapeutic Good Administration (TGA) approved and are available over the counter at most pharmacies, supermarkets and health food outlets.

The electrical equipment used to record your brain activity meets Australian Safety Standards and will be recorded in a Body Protected environment.

The fMRI facility at the Brain Research Institute on the Austin and Repatriation Hospital campus also meets hospital biomedical standards. The fMRI procedure involves other potential risks. You will be screened for the presence of metal within or on your body (such as aneurism clips bone screws), and presence of electronic devices, such as heart pacemakers that may be damaged by the high magnetic field. You will not be selected for the fMRI experiments if you are pregnant.

6. Privacy, Confidentiality and Disclosure of Information

Information, which personally identifies your data, will be kept at the Brain Sciences Institute in strict confidence. If you so request, you may have access to your own data and blood reports. This data will be kept for at least five years after the findings are published. Then the data will be fully de-identified. None of the participants in this study will be individually identified in any resulting publications or reports. Volunteers are entirely free to discontinue participation at any time, or to decline to answer particular questions. The data obtained may be used as a comparison group for future experimentation, but only as group data for statistical purposes and without identification.

7. New Information Arising During the Project.

On extremely rare occasions significant brain abnormalities are found even in completely healthy people. If an abnormality is found, the MRI specialist will contact the principal researcher and advise whether a medical follow-up is needed. If this occurs, the person(s) supervising the research will stop your participation and will contact you to discuss your medical options. In all cases, you will be advised to contact your general practitioner and the researchers will send you your individual results and your fMRI scans (if applicable).

8. Results of Project.

Group data may be published in an academic journal at the completion of this project; however anonymity is assured. In the case of individual data, such as an fMRI image, code numbers will be used and you will be in no way identifiable.

9. Further Information or Any Problems

If you require further information or if you have any problems concerning this project (for example, side effects), you can contact the following:

Name: Prof David Crewther

Position: Deputy Director (Research)

Department: Brain Sciences Institute, Faculty of Life and Social Sciences

Address: 400 Burwood Road, Hawthorn, Victoria, 3122

Phone: 9214 5877

10. Other Issues

Potential conflict of interest

Novasel Australia is providing partial funding for this study as part of a collaborative agreement with Swinburne University (2007). In addition, Novasel Australia will also commit funding to this project through an Australian Research Council (ARC) Linkage grant (LP0884003) and collaborative research agreement with Swinburne University (April 1st 2009). Novasel markets a range of fish oil supplements. Whilst Novasel has full rights of ownership to research data/intellectual property generated in this study, Novasel has agreed by way of a contract not to restrict publication of study findings by researchers.

Complaints or questions

If you have any complaints about any aspect of the project, the way it is being conducted or any other questions about your rights as a research participant, then you may contact:

Contact: Research Ethics Officer, Human Research Ethics Committee

Institute: Swinburne University of Technology

Address: PO Box 218, Hawthorn VIC 3122

Phone: 03 9214 5218

You will need to tell “The Research Ethics Officer” the name of the principal researcher given in section 9 above.

11. Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not take part, or to take part and then withdraw, will not affect your relationship with researchers or staff at Swinburne University.

Before you make your decision, a member of the research team will be available to answer any question you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

12. Ethical Guidelines

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)* co-produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The Human Research Ethics Committee of this Institution has approved the ethical aspects of this research project.

13. Reimbursement for your costs.

You will be paid $100 for your participation in the 3-month supplementation study, and $150 if you choose to participate in the 6-month follow-up study.

SWINBURNE UNIVERSITY OF TECHNOLOGY

Full Project Title: Effects of fish oil on brain performance and cardiovascular measures in a population of healthy volunteers and in a population of adults presenting with Attention Deficit Hyperactivity Disorder (ADHD) symptoms

I have read, or have had read to me in my first language, and I understand the Participant Information, dated / /

I freely agree to participate in this project according to the conditions on the Participant Information.

I will be given a copy of the Participant Information and Consent Form to keep.

The researcher has agreed not to reveal my identity and personal details if information about the project is published or presented in any public form.

[OPTIONAL] Are you interested in taking part in the neuroimaging investigation? If you agree, this will mean that you may be randomly selected to take part in this part of the study.

Yes, I agree ☐ No, I do not agree ☐

[OPTIONAL] Are you interested in taking part in the 6-month fish oil follow-up study? If you agree this will mean that we will keep a record of your contact details:

Yes, I agree ☐ No, I do not agree ☐

Participant's Name (printed)_____

Signature: Date:

Researcher's Name (printed)_____

Signature: Date:

Note: All participants signing the Consent Form must date their own signature.

[OPTIONAL] Do you agree to be contacted with regard to participation on future research studies being conducted at the Brain Sciences Institute? If you agree this will mean that we will keep a record of your contact details:

Yes, I agree ☐ No, I do not agree ☐

SWINBURNE UNIVERSITY OF TECHNOLOGY

Revocation of Consent Form

Full Project Title: The effects of fish oils on cognitive performance and brain function

I hereby WITHDRAW my consent to participate in the research proposal described above and understand that such withdrawal WILL NOT jeopardise any treatment or my relationship with Swinburne University or the researchers involved in this study.

Participant's Name (printed)_____

Signature: Date:

Name of Witness (printed)_____

Signature: Date:

Appendix ii: Health and demographic questionnaire for the omega-3 supplementation study.

Demographic Questions

1. Age: ____

2. Height:

3. Weight

4. Gender:

- Male
- Female

5. Handedness:

- Left
- Right

6. Education level (please indicate highest level completed.)

- Primary School
- Secondary School
  - what year did you complete……
- TAFE
- Undergraduate Degree
- Postgraduate Degree

7. How often do you eat fish?

- Never

- Once a month
- Once a week
- More than once per week

8. How often do you eat fruits and vegetables?

- Several times a day
- Once a day
- 2-3 times a week
- Once a week
- Very rarely

9. How often do you eat takeaways?

- Never
- Once a month
- Once a week
- More than once per week

9. How often do you eat chips and/or chocolate and/or fried foods?

- Never
- Once a month
- Once a week
- More than once per week

8. Are you currently taking any other health supplements?

Please list and indicate how often you take it

Product # 1……

- Never
- Once a month
- Once a week
- More than once per week

Product # 2……..

- Never
- Once a month
- Once a week
- More than once per week
- Daily

Other health supplements?……..

9. Are you currently taking any medications?

Please list and indicate how often you take it

Product # 1……

- Never
- Once a month
- Once a week
- More than once per week

Product # 2……..

- Never
- Once a month
- Once a week
- More than once per week
- Daily

10. How often do you exercise?

- Never
- Once a month
- Once a week
- More than once per week
- Daily

What kind of physical activity?.....

How long for?.....

11. Are you currently a smoker? Yes / No (please circle)

If no, did you previously smoke? Yes / No

How many years were you a smoker? ….

Light / moderate / heavy (please circle)

If yes, how often do you smoke?

- Once a month
- Once a week
- More than once per week
- Once daily
- Less than 5 cigarettes per day
- 5-10 cigarettes per day
- More than 10 cigarettes per day
- More than 20 cigarettes per day

Appendix iii: Depression Anxiety and Stress Scale (DASS)

DASS

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all

1 Applied to me to some degree, or some of the time

2 Applied to me to a considerable degree, or a good part of time

3 Applied to me very much, or most of the time

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, lifts, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Reminder of rating scale:

0 Did not apply to me at all

1 Applied to me to some degree, or some of the time

2 Applied to me to a considerable degree, or a good part of time

3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

Appendix iv: Safety check form for fMRI participants.

MRI SAFETY CHECK – Request for further information

Brain Research Institute

Please complete this form if the answers given on the MR safety checklist have generated a need for further information.

SECTION A: To be completed by researcher.

Study: _____ **SB Number:** _____

Researcher: _____ **Contact Phone Number:** _____

Name of patient: _____

DOB: ____/____/____ **Contact Phone Number:** _____

Contact Address: _____

P/Code: _____ **Date of Scan:** _____

1. OPERATION / PROCEDURE _____
2. SURGEON _____ 3. HOSPTIAL _____
4. DATE OF PROCEDURE: ____/____/____
5. IMPLANT NAME: _____
6. MANUFACTURER OF IMPLANT _____
7. OPERATION NOTES ATTACHED: YES / NO

SECTION B: To be completed by BRI.

8. DETAILS OF IMPLANT _____

_____

_____

_____

9. COPY OF ITEM LABEL ATTACHED: YES / NO
10. RELEVANT DOCUMENTATION ATTACHED YES / NO
11. SAFE @ 3T ACCORDING TO MR SAFETY GUIDELINES YES / NO
12. SAFE @ 3T ACCORDING TO MANUFACTURER'S INFORMATION YES / NO

Further details:

_____

_____

_____

Please sign and date if this Implant or Device has been determined MR Safe@ 3T by BRI MR Staff for the purpose of the above research scan:

Name: _____ **Signed** _____ **Date:** ____/____/____

Name: _____ **Signed** _____ **Date:** ____/____/____

Brain Research Institute Pty Ltd ABN 95 448 150 232
Neurosciences Building Austin Health, 300 Waterdale road, Heidelberg West 3081.
Ph 9496 4137 Fax 9496 4071. Email: bri@brain.org.au

Brain Research Institute

MAGNETIC RESONANCE IMAGING (MRI) SCREENING FORM

Name: _____

Study: _____ **SB Number**_____

DOB: ____/____/____ **Weight:** _____kg **Height:** _____

Gender: Male / Female **Contact Phone Number:** _____

Contact Address: _____

_____ P/Code: _____

1. Have you ever had surgery or an operation? **No / Yes**
   If yes, please indicate the date and type of surgery _____
2. Have you ever had a diagnostic imaging study or examination (MRI, CT, X-ray)? **No / Yes**
3. Have you ever had:
   - Epilepsy/Seizures: **No / Yes**
   - Brain Infection: **No / Yes**
   - Febrile Convulsions: **No / Yes**
   - Psychiatric Disease: **No / Yes**
   - Head Injury: **No / Yes**
   - Other health issues: **No / Yes**
4. Do you currently have any neurological symptoms (e.g. weakness, double vision)? **No / Yes**
5. Are you on any medication? **No / Yes**
6. Are you allergic to any medication? **No / Yes**
7. Do you have any history of renal disease? **No / Yes**

For female patients:

8. Date of last menstrual period: ____/____/____ or Postmenopausal? **No / Yes**
9. Are you pregnant or experiencing a late menstrual period? **No / Yes**

Further details:

_____

_____

_____

_____

For Office Use Only

Status: Control /Patient Time In: ____:____ Time Out: ____:____

Notes: _____

_____

_____

_____

Brain Research Institute Pty Ltd ABN 95 448 150 232
Neurosciences Building Austin Health, 300 Waterdale road, Heidelberg West 3081.
Ph 9496 4137 Fax 9496 4071. Email: bri@brain.org.au

Certain implants, devices, or objects may interfere with the MR procedure. To help us to determine your suitability for an MRI scan and to ensure your safety, please complete the following checklist carefully.

Brain Research Institute

Please indicate if you have any of the following:

ITEM/DEVICE	NO	YES
Aneurysm Clips		
Cardiac Pacemaker / Defibrillator		
Neuro-stimulation system		
Implanted electrical device		
Vascular Surgery Clips		
Artificial Heart Valve		
Intra-ventricular or Spinal Shunt		
Metallic Stent / Filter or Coil		
Surgical staples, metallic sutures or metallic plates		
Eye Implant or Eye Operation		
An injury to your eye involving metal fragments?		
Cochlear or other Ear Implant		
Orthopaedic Devices (screws/rods/pins/plates/nails etc.)		
Any metallic fragment or foreign body		
Dentures (false teeth)		
Hearing Aid (Please remove before entering MR room)		
Piercing Jewellery		
Tattoo or Permanent Makeup		
Any other surgical procedures / operations / implants?		
If yes provide details:		

IMPORTANT INSTRUCTIONS

Before entering the MR room, you must remove all metallic objects including hearing aids, dentures, glasses, partial plates, mobile phones, pagers, watch, hairpins, safety pins, jewellery, body piercings, keys, coins, bank cards, magnetic strip cards, & pens.

Please consult the MRI Radiographer if you have any questions or concerns BEFORE you enter the MR room.

NOTE: You will be required to wear hearing protection during the MRI procedure.

I confirm that the above information is correct to the best of my knowledge. I have read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signed: _____

Parent or Guardian if under 18: _____

Witnessed: _____ Date:_____

Brain Research Institute Pty Ltd ABN 95 448 150 232
Neurosciences Building Austin Health, 300 Waterdale road, Heidelberg West 3081.
Ph 9496 4137 Fax 9496 4071. Email: bri@brain.org.au

Brain Research Institute

MRI CONSENT FORM

Research studies carried out at BRI are designed to improve our knowledge and are not designed for clinical purposes.

After your scan a specialist will review the pictures; however this will not be done on the day of your study. You should be aware that sometimes even in completely healthy people, minor abnormalities are found. On the other hand, because the pictures are taken for a specific research purpose, not all abnormalities are necessarily seen. On extremely rare occasions, we might find an abnormality that is significant and which may need to be investigated further. If a significant abnormality is found, we will contact the researcher directly involved in your study.

Although such a finding is extremely unlikely, please take the time to consider carefully what it would mean to you. It would be entirely your choice as to what you might do with any such information. However, knowledge of an abnormality may affect your ability to do such things as work in certain professions, obtain life or health insurance, etc. If you do not want to know, then you are under no obligation to participate in this part of the study.

I confirm that I have read and understand the above information and that I have the opportunity to ask questions. I confirm that I agree to have an MRI scan as part of the research study "Effects of fish oil on brain efficiency in a healthy adult population and in a population of adults presenting with Attention Deficit Hyperactivity Disorder (ADHD) symptoms".

Signed: Date:

Parent or Guardian if under 18: Date:

Witnessed: Date:

Do you agree to be contacted with regard to participation on future research studies being conducted at the Brain Sciences Institute (BSI)? If you agree this will mean that we will keep a record of your contact details:

Yes, I agree ☐ No, I do not agree ☐

Signed: Date:

Parent or Guardian if under 18: Date:

Witnessed: Date:

Brain Research Institute Pty Ltd ABN 95 448 150 232
Neurosciences Building Austin Health, 300 Waterdale road, Heidelberg West 3081.
Ph 9496 4137 Fax 9496 4071. Email: bri@brain.org.au

Appendix v: Telephone screening questionnaire for ADHD participants

TELEPHONE SCREENING QUESTIONNAIRE- ADHD

QUESTION	EXCLUSION CRITERIA
How old are you?	Less than 18, older than 35
Are you currently taking fish oil supplements?	If yes, then a 3 month washout is necessary.
Can you commit to a three month supplementation period?	If no, then cannot be a participant
Are you interested in extending this to a six month period?	If yes, consider for longer study
Are you willing to attend two 2-hour testing sessions at Swinburne University in Hawthorn?	If no, then cannot be a participant
Would you consent to two blood tests?	If no, cannot be a participant
Would you consent to having two fMRI testing sessions at the Austin hospital?	If yes, add to fMRI group
Do you have a history of neurological trauma or disease?	If yes, cannot be a participant
Do you have a history of psychiatric disorders?	If yes, cannot be a participant
Do you have diabetes, food intolerances or allergies?	If yes, then check to see if they can participate
Do you have a previous or current diagnosis of attention deficit hyperactivity disorder?	If no, consider for control group
If yes to the above question, are you taking any medication for this disorder?	If yes, then may not be suitable
Do you feel you have symptoms of attention deficit hyperactivity disorder?	If yes, don't put in control group but consider for ADHD group.

OTHER QUESTIONS

Can we please have some contact details?

When are you available?

Appendix vi: Telephone screening questionnaire for control participants

TELEPHONE SCREENING QUESTIONNAIRE- CONTROLS

QUESTION	EXCLUSION CRITERIA
How old are you?	Less than 18, older than 35
Are you currently taking fish oil supplements?	If yes, then a 3 month washout is necessary.
Can you commit to a three month supplementation period?	If no, then cannot be a participant
Are you interested in extending this to a six month period?	If yes, consider for longer study
Are you willing to attend two 2-hour testing sessions at Swinburne University in Hawthorn?	If no, then cannot be a participant
Would you consent to two blood tests?	If no, cannot be a participant
Would you consent to having two fMRI testing sessions at the Austin hospital?	If yes, add to fMRI group
Do you have a history of neurological trauma or disease?	If yes, cannot be a participant
Do you have a history of psychiatric disorders?	If yes, cannot be a participant
Do you have diabetes, food intolerances or allergies?	If yes, then check to see if they can participate
Have you had a previous diagnosis of attention deficit hyperactivity disorder?	If yes, don't put in control group but consider for ADHD group.
Do you feel you have symptoms of attention deficit hyperactivity disorder?	If yes, don't put in control group but consider for ADHD group.

OTHER QUESTIONS

Can we please have some contact details?

When are you available?

Appendix vii: Advertisement for ADHD participants.

Have you previously been diagnosed with ADHD?

Do you suspect you may have ADHD but have never been diagnosed?

If yes, we need your help!

Participants are required for a study assessing the effects of Fish oil supplementation on ADHD symptoms. If you are currently on ADHD medication, your participation is still welcome.

To participate in the study you will need to be:

- Aged 18-40
- Not currently taking a fish oil supplement
- No history of neurological disease, diabetes or food intolerance/allergies

The study will use a selection of cognitive and cardiovascular tests to assess your cognition and brain function with and without fish oil supplementation. You will be required to attend two, 2-hour testing sessions at the Brain Sciences Institute (Swinburne University) and have two blood tests. Some of the participants will be invited to complete two brain imaging tests (fMRI) at the Brain research Institute, Austin Hospital.

You will receive $100 for your participation in this study.

If you have queries regarding this study and/or are interested in participating please contact us on 92144542 or by email:

Isabelle Bauer

ibauer@swin.edu.au

Laura Sellick

lsellick@swin.edu.au

Appendix viii: Advertisement for Control participants.

Participants Required for

Fish Oil Supplementation Study

Healthy participants are required for a study assessing the effects of Fish oil supplementation on cognition and brain function.

To participate in the study you will need to be:

- Aged 18-35
- Not currently taking a fish oil supplement
- No history of neurological disease, psychiatric disorders, diabetes or food intolerance/allergies

The study will use a selection of psychometric, cardiovascular, electrophysiological and blood tests to assess your cognition and brain function with and without fish oil supplementation. You will be required to attend two, 2-hour sessions at the Brain Sciences Institute (Swinburne University) and have two blood tests. Some of

the participants will be invited to complete two brain imaging tests (fMRI) at the Brain research Institute, Austin Hospital.

You will receive $100 for your participation in this study.

If you have queries regarding this study and/or are interested in participating please contact us on 92148291 or by email:

Isabelle Bauer
ibauer@swin.edu.au

Laura Sellick
lsellick@swin.edu.au

Appendix ix: Ethics clearance

SWIN BUR NE
SWINBURNE UNIVERSITY OF TECHNOLOGY

Swinburne University of Technology
Human Research Ethics Committee (SUHREC)
Certificate of Ethics Clearance

SUHREC Project 2009/186
Effects of fish oil on brain performance and cardiovascular measures in a population of health volunteers and in a population of adults presenting with Attention Deficit

Chief Investigator/Supervisor:	Prof David Crewther
Main Student Investigator(s):	Miss Isabelle Bauer / Miss Rachel Gold
Duration Approved:	20/11/2009 To 20/11/2011

This is to certify that the above project has been given ethics clearance in accordance with the current *National Statement on Ethical Conduct in Human Research*. The standard conditions and any special conditions for on-going ethics clearance are here printed.

All human research activity undertaken under Swinburne auspices must conform to Swinburne and external regulatory standards, including the above-mentioned *National Statement* and with respect to secure data use, retention and disposal.

The named Swinburne Chief Investigator/Supervisor remains responsible for any personnel appointed to or associated with the project being made aware of ethics clearance conditions, including research and consent procedures or instruments approved. Any change in chief investigator/supervisor requires timely notification and SUHREC endorsement.

The above project has been approved as submitted to date for ethical review by or on behalf of SUHREC. Amendments to approved procedures or instruments ordinarily require prior ethical appraisal/ clearance. SUHREC must be notified immediately or as soon as possible thereafter of (a) any serious or unexpected adverse effects on participants and any redress measures; (b) proposed changes in protocols; and (c) unforeseen events which might affect continued ethical acceptability of the project.

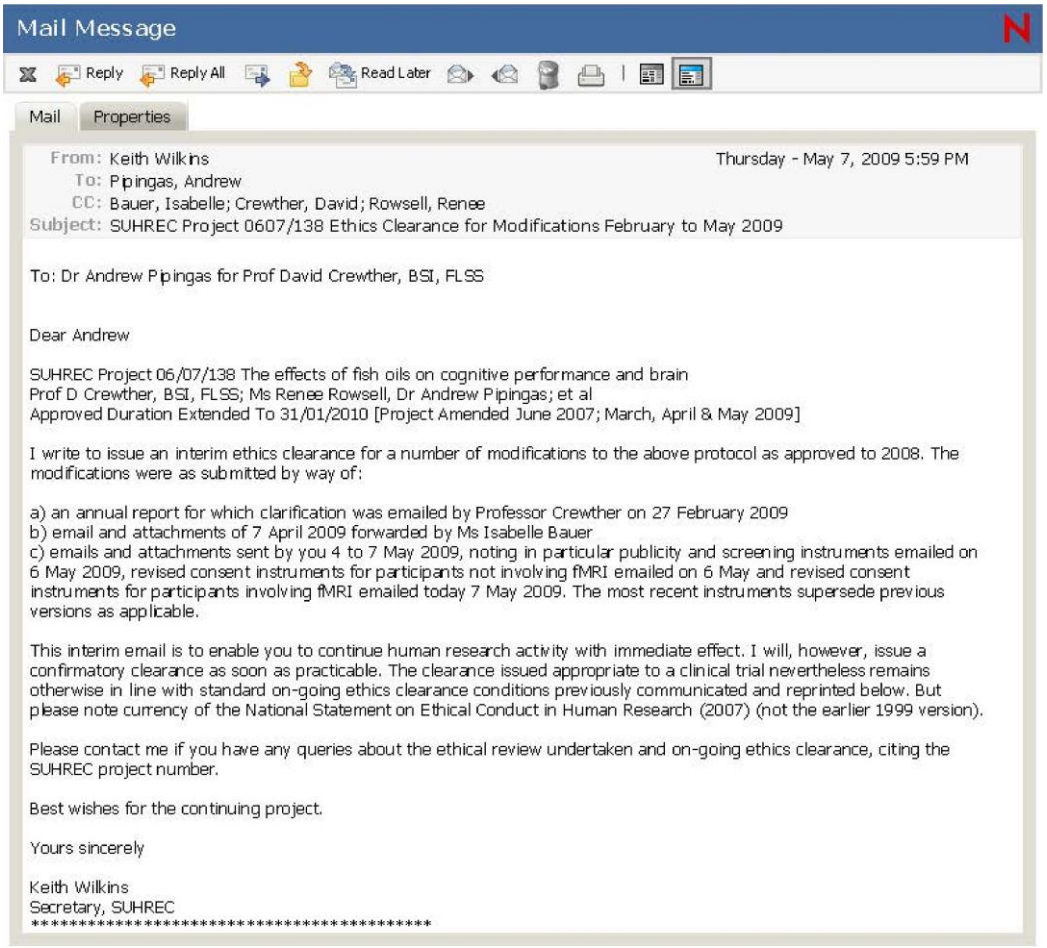
At a minimum, an annual report on the progress of the project is required as well as at the conclusion (or abandonment) of the project.

A duly authorised external or internal audit of the project may be undertaken at any time.

The SUHREC project number and title should be cited in any communication.

Keith Wilkins
Secretary, SUHREC and Research Ethics Officer
03/12/2009

Swinburne Research
John Street Hawthorn
Victoria 3122 Australia
PO Box 218 Hawthorn
Victoria 3122 Australia
Telephone +61 3 9214 5223
Facsimile +61 3 9214 5267
www.swinburne.edu.au/research
CRICOS Provider:00111D
ABN 13 628 586 699



Mail Message

Reply Reply All Read Later

Mail Properties

From: Keith Wilkins Thursday - May 7, 2009 5:59 PM
To: Pipingas, Andrew
CC: Bauer, Isabelle; Crewther, David; Rowsell, Renee
Subject: SUHREC Project 0607/138 Ethics Clearance for Modifications February to May 2009

To: Dr Andrew Pipingas for Prof David Crewther, BSI, FLSS

Dear Andrew

SUHREC Project 06/07/138 The effects of fish oils on cognitive performance and brain
Prof D Crewther, BSI, FLSS; Ms Renee Rowsell, Dr Andrew Pipingas; et al
Approved Duration Extended To 31/01/2010 [Project Amended June 2007; March, April & May 2009]

I write to issue an interim ethics clearance for a number of modifications to the above protocol as approved to 2008. The modifications were as submitted by way of:

a) an annual report for which clarification was emailed by Professor Crewther on 27 February 2009
b) email and attachments of 7 April 2009 forwarded by Ms Isabelle Bauer
c) emails and attachments sent by you 4 to 7 May 2009, noting in particular publicity and screening instruments emailed on 6 May 2009, revised consent instruments for participants not involving fMRI emailed on 6 May and revised consent instruments for participants involving fMRI emailed today 7 May 2009. The most recent instruments supersede previous versions as applicable.

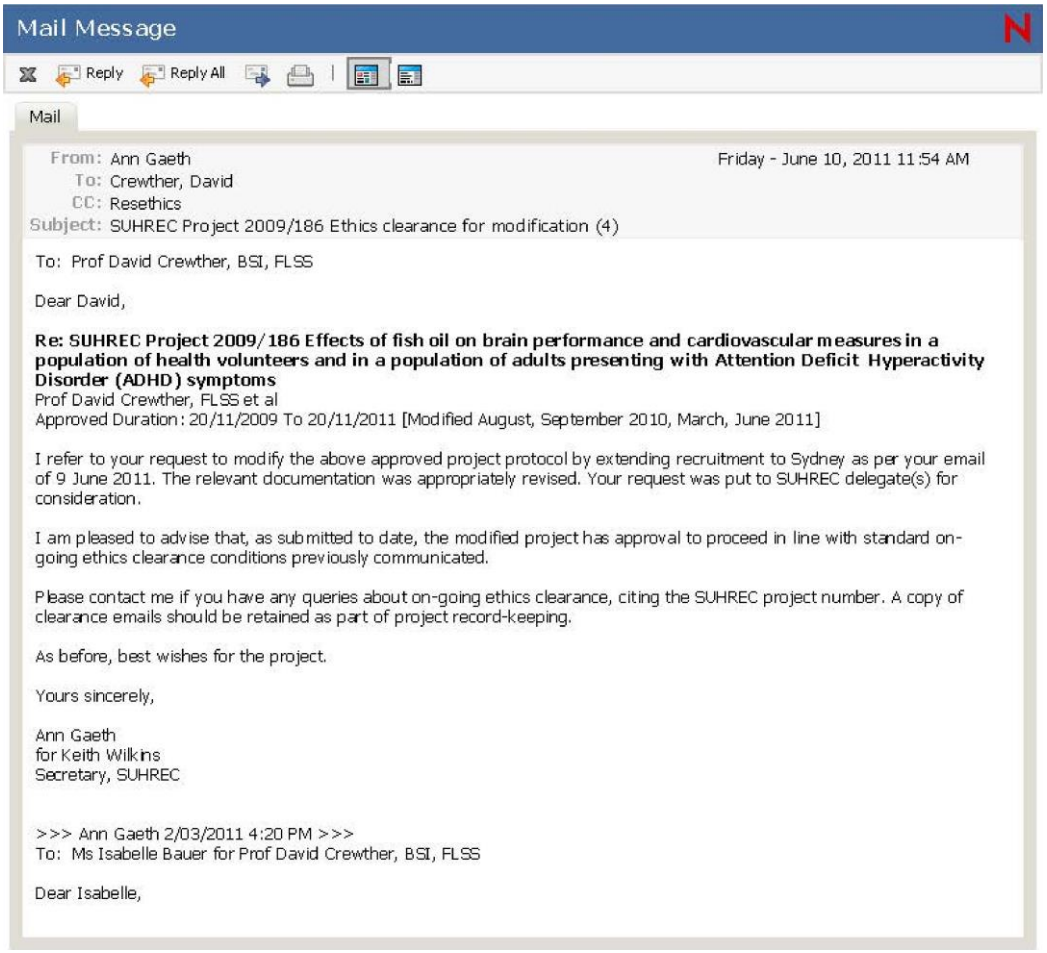
This interim email is to enable you to continue human research activity with immediate effect. I will, however, issue a confirmatory clearance as soon as practicable. The clearance issued appropriate to a clinical trial nevertheless remains otherwise in line with standard on-going ethics clearance conditions previously communicated and reprinted below. But please note currency of the National Statement on Ethical Conduct in Human Research (2007) (not the earlier 1999 version).

Please contact me if you have any queries about the ethical review undertaken and on-going ethics clearance, citing the SUHREC project number.

Best wishes for the continuing project.

Yours sincerely

Keith Wilkins
Secretary, SUHREC
****



Mail Message

Reply Reply All

Mail

From: Ann Gaeth Friday - June 10, 2011 11:54 AM
To: Crewther, David
CC: Resethics
Subject: SUHREC Project 2009/186 Ethics clearance for modification (4)

To: Prof David Crewther, BSI, FLSS

Dear David,

Re: SUHREC Project 2009/186 Effects of fish oil on brain performance and cardiovascular measures in a population of health volunteers and in a population of adults presenting with Attention Deficit Hyperactivity Disorder (ADHD) symptoms
Prof David Crewther, FLSS et al
Approved Duration: 20/11/2009 To 20/11/2011 [Modified August, September 2010, March, June 2011]

I refer to your request to modify the above approved project protocol by extending recruitment to Sydney as per your email of 9 June 2011. The relevant documentation was appropriately revised. Your request was put to SUHREC delegate(s) for consideration.

I am pleased to advise that, as submitted to date, the modified project has approval to proceed in line with standard on-going ethics clearance conditions previously communicated.

Please contact me if you have any queries about on-going ethics clearance, citing the SUHREC project number. A copy of clearance emails should be retained as part of project record-keeping.

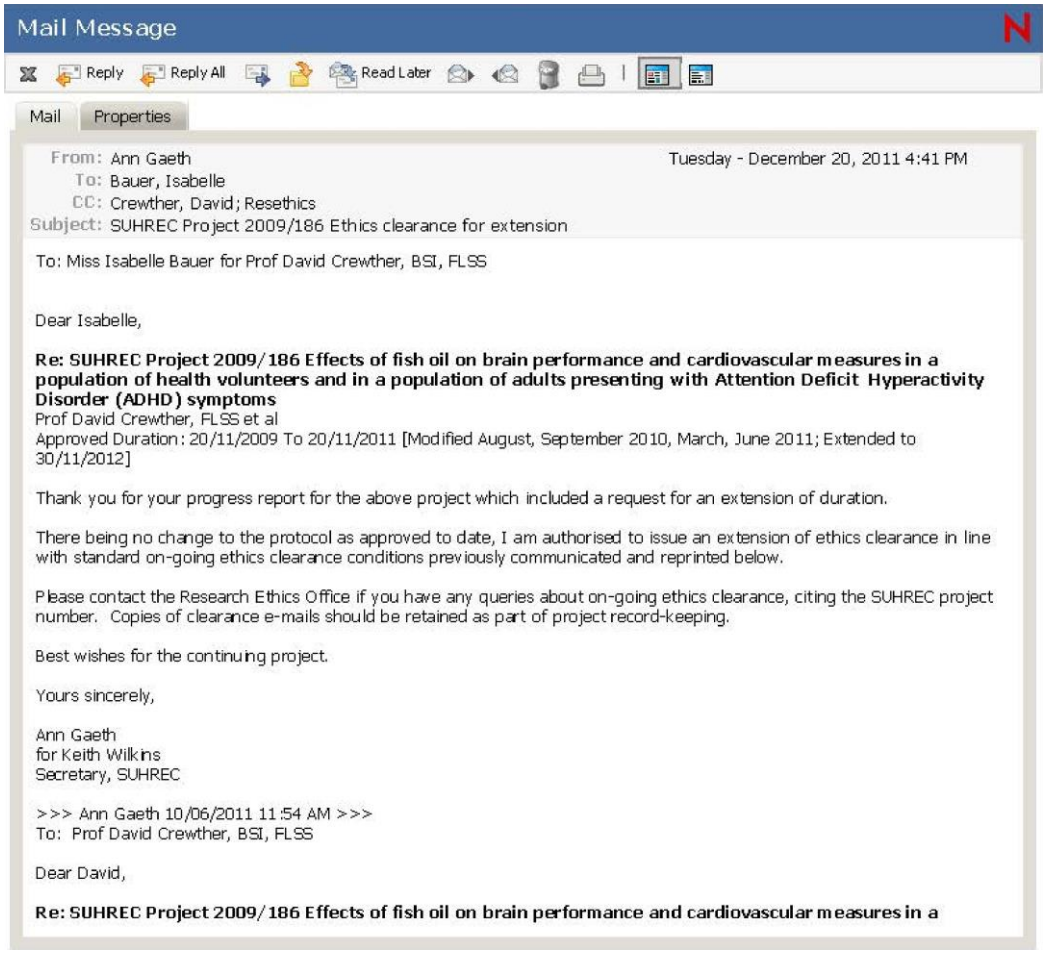
As before, best wishes for the project.

Yours sincerely,

Ann Gaeth
for Keith Wilkins
Secretary, SUHREC

>>> Ann Gaeth 2/03/2011 4:20 PM >>>
To: Ms Isabelle Bauer for Prof David Crewther, BSI, FLSS

Dear Isabelle,



Mail Message

Reply Reply All Read Later

Mail Properties

From: Ann Gaeth Tuesday - December 20, 2011 4:41 PM
To: Bauer, Isabelle
CC: Crewther, David; Resethics
Subject: SUHREC Project 2009/186 Ethics clearance for extension

To: Miss Isabelle Bauer for Prof David Crewther, BSI, FLSS

Dear Isabelle,

Re: SUHREC Project 2009/186 Effects of fish oil on brain performance and cardiovascular measures in a population of health volunteers and in a population of adults presenting with Attention Deficit Hyperactivity Disorder (ADHD) symptoms
Prof David Crewther, FLSS et al
Approved Duration: 20/11/2009 To 20/11/2011 [Modified August, September 2010, March, June 2011; Extended to 30/11/2012]

Thank you for your progress report for the above project which included a request for an extension of duration.

There being no change to the protocol as approved to date, I am authorised to issue an extension of ethics clearance in line with standard on-going ethics clearance conditions previously communicated and reprinted below.

Please contact the Research Ethics Office if you have any queries about on-going ethics clearance, citing the SUHREC project number. Copies of clearance e-mails should be retained as part of project record-keeping.

Best wishes for the continuing project.

Yours sincerely,

Ann Gaeth
for Keith Wilkins
Secretary, SUHREC

>>> Ann Gaeth 10/06/2011 11:54 AM >>>
To: Prof David Crewther, BSI, FLSS

Dear David,

Re: SUHREC Project 2009/186 Effects of fish oil on brain performance and cardiovascular measures in a