Northumbria Research Link

Citation: Jackson, Philippa (2010) Biobehavioural and cerebral hemodynamic effects of omega-3 polyunsaturated fatty acids in healthy individuals. Doctoral thesis, Northumbria University.

This version was downloaded from Northumbria Research Link: https://nrl.northumbria.ac.uk/id/eprint/2386/

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: http://nrl.northumbria.ac.uk/policies.html

Northumbria University NEWCASTLE

CUSTOMER SERVICE EXCELLENCE CSE

UniversityLibrary

Biobehavioural and cerebral hemodynamic effects of omega-3 polyunsaturated fatty acids in healthy individuals

PHILIPPA ALICE JACKSON

A thesis submitted in partial fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy

Research undertaken in the School of Psychology and Sports Science, University of Northumbria

March 2010

ABSTRACT

The omega-3 polyunsaturated fatty acids (n-3 PUFAs) are a unique class of fatty acids that cannot be manufactured by the body, and must be acquired via dietary sources. In the UK, as well as in other Western nations, these 'essential' fatty acids are consumed in quantities that fall below government guidelines. This thesis examined the relationship between n-3 PUFAs and cognitive function and mood in healthy children (8-10 years) and adults (18-35 years), with a view to evaluate their efficacy for cognitive and mood enhancement in these populations. A second aim was to evaluate the effects of n-3 PUFAs on cerebral hemodynamics, a novel line of enquiry.

Chapters 2 and 4 describe novel intervention studies that assessed the effects of n-3 PUFA supplements on cognitive function and mood in healthy children and adults, respectively. In Chapter 3, the relationship between peripheral PUFA concentrations, a correlate of dietary PUFA intake, and cognitive and function and mood was examined for the first time in healthy adults. Chapter 5 describes a pilot trial in which Near Infrared Spectroscopy (NIRS) imaging technique was applied to investigate the cerebral hemodynamic effects of n-3 PUFA supplements. The results of this study were explored in more detail in Chapter 6, with the additional inclusion of parallel cognitive measures.

Most notably, the behavioural data from the intervention studies described herein do not support the use of n-3 PUFA supplements for cognitive and mood enhancement in healthy children and adults not consuming appreciable amounts of oily fish. However, the results do suggest that supplementation with dietary n-3 PUFAs has an impact on peripheral fatty acid status and cerebral hemodynamics in healthy adults. Taken together, these findings suggest that, in healthy, cognitively intact individuals, short-term use of n-3 PUFA supplements has a minimal effect on behaviour; the impact of long-term n-3 PUFA dietary intake or supplement use over the course of the entire lifespan on behaviour should be addressed further.

List of Contents

Abstract i

List of Tables and Figures vi

Acknowledgements viii

Author's Declaration ix

CHAPTER 1. INTRODUCTION 1
1.1 Background 1
1.2 Essential fatty acid nomenclature, structure and metabolism 2
1.3 Dietary sources, consumption and cellular incorporation of n-3 PUFAs 3
1.4 The functions of n-3 PUFAs 4
1.4.1 Incorporation of DHA maintains neuronal cell membrane fluidity and function 5
1.4.2 DHA and EPA as precursors of secondary signalling molecules 6
1.4.3 N-3 PUFAs modulate gene expression 8
1.4.4 N-3 PUFAs modulate neurotransmission 8
1.4.5 N-3 PUFAs are neuroprotective 9
1.4.6 The cardiovascular effects of n-3 PUFAs 10
1.4.7 Summary of the functions of n-3 PUFAs 11
1.5 The behavioural effects of n-3 PUFAs 12
1.5.1 Animal evidence 12
1.5.2 Human evidence 14
1.5.2.1 The role of n-3 PUFAs in neuropsychiatric conditions 15
1.5.2.2 The role of n-3 PUFAs in neurodevelopmental disorders 25
1.5.2.3 N-3 PUFA supplementation and behaviour in healthy individuals 27
1.5.3 Summary of the behavioural effects of n-3 PUFAs 32
1.6 Rationale 33

CHAPTER 2. COGNITIVE AND MOOD EFFECTS OF 8 WEEKS' SUPPLEMENTATION WITH 400 MG OR 1000 MG DHA IN HEALTHY CHILDREN AGED 10-12 YEARS 35
2.1 Introduction 35
2.2 Materials and Methods 38
2.2.1 Design 38
2.2.2 Participants 38
2.2.3 Treatments 39
2.2.4 Cognitive and Mood Measures 40
2.2.4.1 Cognitive Drug Research (CDR) Battery 40
2.2.4.2 Internet Battery 42
2.2.4 Procedure 45
2.2.4.1 Primary assessment 45
2.2.4.2 Secondary assessment 46
2.2.4.3 Statistical methods 46
2.3 Results 48

2.3.1 CDR battery 48
2.3.2 Internet Battery 48
2.4 Discussion 53

CHAPTER 3. THE RELATIONSHIP BETWEEN SERUM PUFAs AND COGNITIVE FUNCTION AND MOOD IN HEALTHY ADULTS (18-35 YEARS) 57
3.1 Introduction 57
3.2 Materials and Methods 60
3.2.1 Design 60
3.2.2 Participants 60
3.2.3 Assessment of cognitive performance 61
3.2.3.1 COMPASS 61
3.2.3.2 Cognitive Demand Battery (CDB) 64
3.2.4 Assessment of mood 65
3.2.5 Procedure 65
3.2.5.1 Blood samples 66
3.2.5.1 Statistical methods 67
3.3 Results 69
3.3.1 Participant characteristics 69
3.3.2. Regression analyses 69
3.4 Discussion 73

CHAPTER 4. COGNITIVE AND MOOD EFFECTS OF DHA-RICH FISH OIL AND EPA-RICH FISH OIL IN HEALTHY ADULTS (18-35 YEARS) 78
4.1 Introduction 78
4.2 Materials and Methods 82
4.2.1 Design 82
4.2.2 Participants 82
4.2.3 Treatments 83
4.2.4 Assessment of cognitive performance and mood 83
4.2.5 Procedure 84
4.2.5.1 Blood samples 85
4.2.5.2 Statistical methods 85
4.3 Results 88
4.3.1 Physiological data and compliance 88
4.3.2 Cognitive performance battery (COMPASS) 89
4.3.3 Cognitive Demand Battery (CDB) 93
4.3.4 Mood 93
4.4 Discussion 94

CHAPTER 5. CEREBRAL HEMODYNAMIC EFFECTS OF DHA-RICH FISH OIL AND EPA-RICH FISH OIL IN HEALTHY YOUNG ADULTS 100
5.1 Introduction 100
5.2 Materials and Methods 104
5.2.1 Design 104
5.2.2 Participants 104
5.2.3 Treatments 104

5.2.4 Physiological Measures 105
5.2.5 Cognitive Tasks 107
5.2.6 Procedure 108
5.2.6.1 Statistical methods 109
5.3 Results 110
5.3.1 NIRS 110
5.3.1.1 Oxyhaemoglobin (O2Hb) 110
5.3.1.2 Deoxyhaemoglobin (HHb) 110
5.3.1.3 Total haemoglobin (THb) 110
5.3.1.2 Cognitive Performance 111
5.4 Discussion 112

CHAPTER 6. THE COGNITIVE AND CEREBRAL HEMODYNAMIC EFFECTS OF DHA-RICH FISH OIL: A DOSE-RANGING STUDY 114
6.1 Introduction 114
6.2 Materials and Methods 117
6.2.1 Design 117
6.2.2 Participants 117
6.2.3 Treatments 118
6.2.4 Near Infrared Spectroscopy 118
6.2.5 Cognitive Assessment 118
6.2.6 Procedure 121
6.2.6.1 Statistical methods 122
6.3 Results 123
6.3.1 Compliance 123
6.3.2 NIRS 123
6.3.2.1 Oxyhaemoglobin (O2Hb) 123
6.3.2.2 Deoxyhaemoglobin (HHb) 123
6.3.2.3 Total haemoglobin (THb) 124
6.3.3 Cognitive Performance 124
6.4 Discussion 128

CHAPTER 7. DISCUSSION 134
7.1 Summary of the objectives of the thesis 134
7.2 The relationship between dietary n-3 PUFAs and cognitive function and mood 135
7.2.1 Children 135
7.2.2 Adults (18-35 years) 137
7.2.2.1 Cognitive function 137
7.2.2.2 Mood 141
7.3 Cerebral hemodynamics 144
7.4 Dietary advice on n-3 PUFA intake 147
7.5 Limitations 149
7.6 Future research 152
7.7 General conclusions 154

APPENDICES **157**
APPENDIX I:Depression, Anxiety and Stress Scales 157
APPENDIX II: Health and diet questionnaire utilised in Chapter 3 159
APPENDIX III: All analysed associations between serum n-3 PUFAs and behavioural outcomes 161
APPENDIX IV: Health and diet questionnaire utilised in Chapter 4 166
APPENDIX V: Family background questionnaire 168
APPENDIX VI: Average scores by group on the cognitive tasks employed in Chapter 5 170
APPENDIX VII: Resting baseline NIRS data collected in Chapter 6 171
APPENDIX VIII: List of journal publications resulting from research conducted as part of this thesis 172

REFERENCES **173**

List of Tables and Figures

CHAPTER 1

Figure 1.1	Metabolic pathways of n-3 and n-6 PUFAs.	3

CHAPTER 2

Table 2.1	Participant demographic details.	38
Figure 2.1	Study day running order of assessments.	46
Table 2.2	Mean data from CDR battery performance outcomes.	49
Table 2.3	Mean data from Internet Battery performance outcomes.	51
Table 2.4	Mean data from Internet Battery mood questionnaire items.	52

CHAPTER 3

Figure 3.1	Assessment schedule of all tasks and measures.	66
Table 3.1	Participant demographic information and serum fatty acid concentrations.	69
Table 3.2	Mean data from cognitive performance and mood outcomes and significant associations between serum concentrations of PUFAs and outcomes.	71

CHAPTER 4

Table 4.1	Participant demographic information and baseline characteristics.	83
Figure 4.1	Baseline and Week 12 assessment schedule.	85
Table 4.2	Mean data from physiological measures at Baseline and Week 12.	88
Table 4.3	Mean data from COMPASS task performance and mood outcomes at Baseline and Week 12.	91
Table 4.4	Mean data from the Cognitive Demand Battery outcome measures at Baseline and Week 12.	93

CHAPTER 5

Figure 5.1	Photon path between NIRS emitter and detector pair.	106
Figure 5.2	Optode placement of the 2-channel NIRS headband (a) and NIRS device (b).	106
Figure 5.3	Mean concentration changes of oxygenated and total haemoglobin by treatment group during the cognitive tasks.	111
Table 5.2	Mean concentration changes of oxyhaemoglobin, deoxyhaemoglobin and total haemoglobin.	111

CHAPTER 6

Table 6.1	Participant demographic details.	118
Table 6.2	Mean concentration changes of oxyhaemoglobin, deoxyhaemoglobin and total haemoglobin.	126
Figure 6.1	Mean concentration changes of oxygenated and total haemoglobin.	127
Table 6.3	Mean data from cognitive task performance by treatment group.	128

APPENDICES

Table III.1	Associations between serum DHA and COMPASS, mood and CDB outcome measures.	163
Table III.2.	Associations between serum EPA and COMPASS, mood and CDB outcome measures.	164
Table III.3.	Associations between serum Total n-3 PUFAs (ALA+DHA+EPA) and COMPASS, mood and CDB outcome measures.	165
Table III.4.	Associations between serum AA and COMPASS, mood and CDB outcome measures.	166
Table III.5.	Associations between serum AA:EPA and COMPASS, mood and CDB outcome measures.	167

Acknowledgements

It is a great pleasure to thank those who contributed to the writing of this thesis.

First and foremost, I would like to offer my sincerest gratitude go to my supervisors Professor David Kennedy, Dr Jonathon Reay and Professor Andrew Scholey. Your guidance and support have been unparalleled and it has been both a privilege and a pleasure working with you. Thank you for imparting your knowledge and for inspiring my research career.

I am indebted to Ginsana SA. Without their sponsorship and provision of the fish oil capsules used in Chapters 4, 5, and 6 this thesis would not have been possible. I am also grateful of the funding and capsules supplied by Martek Biosciences for the study described in Chapter 2.

A special thank you to Michael Deary for all your hard work conducting the fatty acid analyses. Thank you also to Anthea Wilde, Ruth Steinberg, Jade Elliott and Joanne Forster for their technical assistance in collecting the blood samples for Chapters 3 and 4.

Thank you also to Jade, Bernadette Robertson, and Crystal Haskell who helped in the data collection for Chapter 2. Additionally I would like to thank Brian Tiplady and Tom Buchanan for their assistance creating the Internet Battery and CDR for providing their battery for use in this study.

I have been blessed with exceptionally supportive colleagues; thank you to everyone in the BPRNC for your friendship, practical help and good times. A big thank you to Crystal and Jade especially for all your advice over the years and above all for being true friends.

I thank my family for instilling in me the value of good education and supporting all my studies thus far.

My heartfelt thanks go to Andi for your patience, understanding and encouragement that have kept me focused and motivated to the end.

Author's Declaration

I declare that:

This work has not been submitted for any other award.

The author and the supervisors worked together on the designing the methodology for each experimental chapter. For these chapters, the data collection, analysis and interpretation was the sole work of the author, except where acknowledged.

The writing of this thesis is the sole work of the author.

Name: Philippa Alice Jackson

Signature:

Date:

CHAPTER 1. INTRODUCTION

1.1 Background

A dietary supplement is a preparation of concentrated nutrients or metabolites such as vitamins, minerals and amino acids that is intended to supplement the diet, often when the nutrient is missing in the diet or is not consumed at sufficient levels. Health benefits such as boosting the immune system or athletic performance and reducing the risk of developing chronic age-related disease are also often listed as motivations for dietary supplementation (Webb, 2006). Further to this, there is increasing interest in optimising brain function with dietary supplements in light of emerging evidence that suggests particular nutrients can influence brain architecture, chemistry and function (Fernstrom, 2000). It is not surprising then that the worldwide market for dietary supplements is forecast to reach $187 billion in 2010 and in the UK the industry was valued at approximately $827 million in 2006, with projected growth at a further 1% each year until 2011 (Themedica, 2009).

Amongst the dietary supplements that have received increasing interest in recent years are the omega-3 polyunsaturated fatty acids (n-3 PUFAs). These fatty acids are naturally enriched in fatty fish such as salmon, mackerel and sardines; the sale of supplements containing n-3 PUFAs represents 20% of the nutritional supplements market share in the UK, second only to multivitamins (Themedica, 2009). A variety of products containing n-3 PUFAs are available, with labels purporting to maintain healthy joints and muscles, cardiovascular health or brain development and function. The marketing of these products is unambiguous, particularly those aimed at supporting brain function (e.g. Eye q mind, Healthspan Brain Boosters, Boots Smart Omega-3 Fish Oil, Vitabiotics Neurozan); however there is very little in the way of human research to indicate what the effects of n-3 PUFA supplements on brain function and behaviour in the general healthy population actually are.

This introduction outlines the many functions of n-3 PUFAs in the body and the impact they may have on brain function and behaviour. The second part of this chapter evaluates the effects of n-3 PUFAs on behaviour by examining the extant

literature in animals and humans. These sections follow a brief overview of the basic structure and metabolism of fatty acids.

1.2 Essential fatty acid nomenclature, structure and metabolism

Humans typically consume about 20 different types of fatty acids in the diet. These can be metabolised for energy, stored in fat deposits or incorporated into cell membrane phospholipids (Surette, 2008). Fatty acids are hydrocarbon chains containing an even number of carbon atoms anywhere in the range of 2-30. The hydrocarbon chain is flanked by a methyl group (CH_3) at one end (the omega end) and a carboxyl group (COOH) at the other. Fatty acids can be grouped into either saturated or unsaturated fatty acids. Saturated fatty acids have single bonds between the carbon atoms and are rigid in nature. Unsaturated fatty acids may have one (monounsaturated) or more (polyunsaturated) double bonds and the position of the first double bond in relation to the omega end determines whether a polyunsaturated fatty acid is termed an omega-3 (n-3) or an omega-6 (n-6) fatty acid. Mammals are capable of manufacturing every fatty acid required for biological processes except for two; namely linoleic acid (LA, n-6) and α-linolenic acid (ALA, n-3). These are termed the 'essential' fatty acids and must be acquired via the diet (Simopoulos, 2000).

LA and ALA are sometimes referred to as 'parent' fatty acids as it is from these that their respective long-chain biologically active metabolites are derived. Arachidonic acid (AA, n-6) is the major metabolite of LA whereas eicosapentaenoic acid (EPA, n-3) and docosahexaenoic acid (DHA, n-3) are the more biologically active metabolites of ALA (Figure 1.1). AA, EPA and DHA are synthesised from their respective precursor parent fatty acids by a series of elongations and desaturations that, despite the fact that the conversion pathways for n-6 and n-3 fatty acids are entirely independent, require the same enzymes at each step. There is also some evidence to suggest that DHA can be 'retro-converted' into EPA, though rates of only 20% have been observed (Gronn et al., 1991). The metabolism of LA and ALA is predominantly carried out in the endoplasmic reticulum of the liver, in certain structures in the central nervous system such as glial cells (Moore, 2001) and the choroid plexus vasculature (Bourre et al., 1997), and has also been observed at low rates in the placenta (Haggarty, 2004).

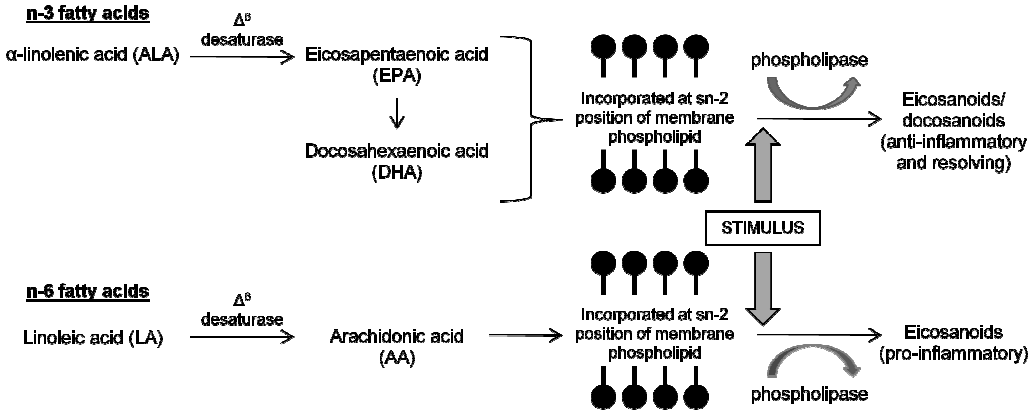

Figure 1.1. Metabolic pathway of n-3 and n-6 PUFAs (adapted from Sanders and Emery, 2003).

The process of conversion of ALA to DHA is extremely inefficient in humans with most studies reporting rates of less than 0.05% (Burdge et al., 2003). Although a considerable amount of variability exists between individuals, it rarely exceeds 9% in women and 4% in men (Burdge and Calder, 2005) and is hindered further by the fact that between 15-35% of the ALA provided by dietary sources is immediately converted to carbon dioxide for energy (Burdge et al., 2002). Further, not only is this process limited in efficiency but as previously mentioned the n-6 and n-3 biosynthetic pathways compete for enzymes; Δ^6 desaturase in particular has a preference to convert ALA to DHA but high dietary intake of LA has been shown to reduce this conversion by 40-50%, resulting in a preferential shift towards the metabolism of LA to AA (Gerster, 1998). So despite the fact that ALA can be used as a source of EPA and DHA; it is more efficient if they are supplied directly via dietary sources.

1.3 Dietary sources, consumption and cellular incorporation of n-3 PUFAs

ALA is highly concentrated in selected seed oils such as linseed and canola and also in the chloroplasts of green leafy vegetables. Algae produce EPA and DHA, and because fish consume algae, they too are rich in these fatty acids which has led to them being referred to as the 'fish oils'. Fatty fish that are rich in DHA and EPA include salmon, mackerel, sardines, trout and fresh tuna. In lesser quantities DHA and EPA can also be found in chicken and their eggs and other livestock if they have been fed a diet enriched with n-3 PUFAs.

The consumption of n-3 PUFAs has been falling gradually over the past 100-150 years; the typical 'Western' diet of today is characterised by a marked decrease in overall fish consumption and increased intake of n-6 PUFAs that are abundant in cooking oils and processed foods (Simopoulos, 2008). There is evidence to suggest that humans evolved on a diet where n-6 and n-3 PUFAs were consumed in approximately equal amounts (1 to 4:1) (Simopoulos, 1991), whereas the consumption ratio of n-6 and n-3 PUFAs in the current Western diet is estimated anywhere between 10: to as much as 25:1 (Simopoulos, 2000). There is also mounting evidence to suggest that decreased dietary intake of n-3 PUFAs, DHA and EPA in particular, is a risk factor for a plethora of different diseases including cardiovascular disease (Mori and Woodman, 2006), inflammatory disease (De Caterina and Basta, 2001) and many neurodevelopmental and psychiatric conditions such as attention deficit hyperactivity disorder (ADHD), dyslexia, depression, schizophrenia and dementia (Bourre, 2005). It follows that for these two n-3 PUFAs to be implicated in such a range of seemingly unrelated conditions, they are likely to influence fundamental processes common to most cells.

Indeed, once consumed (or metabolised) DHA and EPA are incorporated at the sn-2 position of cellular membrane phospholipids in every type of tissue, where they compete for incorporation at the same position with AA (Calder, 2006a). Under certain conditions, DHA and EPA (and AA) are released from the cell membrane by the action of several phospholipases (Farooqui et al., 1997), where they are metabolised further to form potent secondary signalling molecules classed as either eicosanoids or docosanoids (Figure 1.1) (Tassoni et al., 2008). The dietary intake of n-3 PUFAs is therefore reflected in the composition of all cell membranes which can impact a number of cellular processes, described below.

1.4 The functions of n-3 PUFAs

N-3 PUFAs are incorporated into the membranes of every single cell in the body. Since they cannot be manufactured *de novo* and the body relies on an external supply provided by dietary sources, it follows that the quantity and type of fatty acids consumed will impact the composition of cellular membranes and in turn influence membrane function (Yehuda et al., 1999). It is this concept that is at the very heart of understanding the different mechanisms by which n-3 PUFAs are believed to

impact brain function and hence behaviour. Although the exact biochemistry of these processes is still under investigation, key functions include maintaining membrane fluidity and the regulation of eicosanoid production, gene expression and neurotransmission. N-3 PUFAs have also been shown to modulate cardiovascular function. Given that the brain relies on a constant supply of blood borne metabolic substrates, modulation of cardiovascular parameters by n-3 PUFAs may potentially have secondary effects on brain function and are therefore also briefly described.

1.4.1 Incorporation of DHA maintains neuronal cell membrane fluidity and function

Communication between neurons relies on the exchange of ions across the cellular membrane, with maximum efficiency occurring at an 'optimal' value where the physical state of the membrane is neither too rigid or too fluid (Yehuda et al., 1999). The structure of the cell membrane varies greatly depending on the fatty acids that make up the hydrophobic 'tail' of the phospholipids. For example, rigid saturated fatty acids allow phospholipids to pack tightly together whereas the insertion of double bonds along the hydrocarbon chain alters the properties of the fatty acid. Therefore, as the degree of unsaturation increases the chain becomes more flexible and starts to 'kink'. DHA, which has six double bonds and is preferentially incorporated at the sn-2 position of the phospholipids phosphatidylethanolamine and phosphatidylserine in particular, can adopt countless looped and helical conformations and thus tight packing of these DHA-rich phospholipids is prevented and consequently increases the fluidity of the membrane (Feller et al., 2002). EPA, possessing five double bonds can also adopt multiple conformations, but the extra double bond present in DHA renders this fatty acid unique and highly specialised, as evidenced by its high density in selected tissues (Stillwell and Wassall, 2003). More specifically, DHA is heavily concentrated in the cerebral frontal cortex of mammals and comprises anywhere between 10-20% of total fatty acids of the brain (McNamara and Carlson, 2006) and represents around 30-40% of the PUFAs found in the retinal rod outer segment (Makrides et al., 1994). Modulation of membrane fluidity in these tissues occurs with dietary manipulation of n-3 PUFAs (Anderson et al., 2005, Connor et al., 1990), and has been shown to affect the activities of membrane bound enzymes and ion channels.

Although the area of cellular enzyme activity is very broad and is still under intense research, two key enzymes associated with brain function that are modulated by n-3 PUFAs are protein kinase C (PKC) and Na^+,K^+-ATPase. PKC describes a family of enzymes that regulate a number of key processes such as transcription, immune responses, cell proliferation and others (Mellor and Parker, 1998), and has also been implicated in learning and memory (Sun and Alkon, 2006). Slater and colleagues (1995) discovered that PKC activity was inversely related to the degree of unsaturation of phosphatidylcholine and phosphatidylserine present in the membrane lipid bilayer, potentially demonstrating how dietary manipulations can have an effect on signal transduction pathways. A positive relationship has also been found to exist between the activation of the enzyme Na^+,K^+-ATPase and the presence of DHA in the surrounding membrane (Turner et al., 2003). Considering around 60% of the brain's energy consumption can be attributed to the action of Na^+,K^+-ATPase, this discovery highlights an important link between DHA and membrane function.

As regards modulation of ion channel function, both DHA and EPA have been shown to inhibit voltage-activated sodium (Kang and Leaf, 1996) and calcium (Xiao et al., 1997) currents by binding directly to the channel proteins that control the passage of these ions. Similarly, these two n-3 PUFAs have been shown to inhibit voltage-activated potassium currents (Seebungkert and Lynch, 2002). Ion channels have an integral role in the successful transmission of neuronal information along the axon as this process is reliant on the passage of electrically charged atoms in and out of the cell via these channels located within the cell membrane (Yehuda et al., 1999).

Taken altogether, it is evident that the structure of cellular membranes, which is modifiable by dietary intake of DHA and EPA, impacts upon cellular processes. In the case of neuronal cell membranes these changes in cellular function may impact upon brain function and hence behaviour.

1.4.2 DHA and EPA as precursors of secondary signalling molecules

In contrast to DHA, there is comparatively little EPA in the brain and it is found to comprise less than 1% of total brain fatty acid composition (de la Presa Owens and Innis, 1999), although it is incorporated into the membranes of all mammalian cells.

EPA is released from membrane phospholipids under a variety of different stimuli and subsequently undergoes enzymatic degradation to become one of several possible lipid-derived messaging molecules (Figure 1.1) (McNamara and Carlson, 2006). These so-called eicosanoids (further categorised as leukotrienes, thromboxanes or prostaglandins) are a class of powerful biological compounds responsible for mediating many aspects of the inflammatory response (Calder, 2006a). Eicosanoids can also be derived from AA upon its release from the cell membrane (see Figure 1.1), but are far more potent than those originating from EPA and tend to be pro-inflammatory (Schmitz and Ecker, 2008). However, increased intake of dietary EPA leads to increased incorporation of these molecules into the membrane phospholipids in a dose response manner and at the expense of membrane incorporation of AA (Calder, 2007). Consequently, there is a shift away from production of pro-inflammatory, vaso-constricting, and platelet aggregating AA-derived eicosanoids, and an increase in production of anti-inflammatory EPA-derived ones (Gibney and Hunter, 1993).

Like eicosanoids, docosanoids are chemical signalling molecules, produced via controlled oxidative degeneration of DHA within or adjacent to the cell membrane (Kidd, 2007). Three classes of docosanoids have been identified—docosatrienes, resolvins and protectins—and have been shown to have neuroprotective qualities. The novel neuroprotectin D1 (NPD1) has been shown to attenuate apoptosis in the presence of oxidative stress and provides protection to neuronal cells in animal models of brain ischemia and neurodegeneration (reviewed in Bazan, 2006). More specifically, in Alzheimer's disease rat models NPD1 repressed the expression of pro-inflammatory β-amyloid activated genes. Moreover, the recently discovered E-series and D-series resolvins, derived from EPA and DHA respectively, have also been identified as having anti-inflammatory properties that are not related to altering lipid mediator profiles (i.e. inhibited production of AA-derived eicosanoids), but by inhibiting the expression of pro-inflammatory cytokine genes such as nuclear factor κ B and/or peroxisome proliferator activated receptor (Calder, 2006b). Taken together, these are the many mechanisms by which DHA and EPA could potentially prevent the occurrence or ameliorate the symptoms of inflammatory diseases linked to n-3 PUFA intake including depression (Das, 2007), ADHD (Richardson, 2006), schizophrenia (Yao and van Kammen, 2004), Alzheimer's disease (Pratico and Trojanowski, 2000), atherosclerosis (von Schacky, 2000) rheumatoid arthritis

(Kremer 2000), inflammatory bowl disease (De Caterina et al., 2000), and possibly some bronchial diseases such as asthma (Belluzzi et al., 2000).

1.4.3 N-3 PUFAs modulate gene expression

The switching on or off of genes, or control of gene expression, allows the cell to control the production of proteins (e.g. enzymes, receptors or structural proteins) as they are required by the cell or throughout the organism (Hawkins, 1991). Diet-induced changes in gene expression have been demonstrated in animals following n-3 PUFA supplementation. DNA microarrays in rats fed either a perilla-enriched (high in ALA) or fish oil-enriched (high in DHA and EPA) chow found that expression levels of a number of genes involved in synaptic plasticity, signal transduction, ion channel formation, energy metabolism and regulatory proteins were modulated compared to controls (Kitajka et al., 2002). In a follow-up experiment it was demonstrated that the ratio of LA to ALA in the experimental diets can further influence the expression of certain genes (Barcelo-Coblijn et al., 2003), highlighting that the ratio of intake of n-6 and n-3 PUFAs can impact cellular function at a very fundamental level. Based on their findings, these authors suggest that the n-3 fatty acid induced alterations in expression of neural genes may be the link between dietary intake and improvements in mental function, though this theory has yet to be supported. Likewise, it is possible that a breakdown in these processes plays a role in the aetiology of some neuropsychiatric or developmental conditions.

1.4.4 N-3 PUFAs modulate neurotransmission

Many neurotransmitters, such as serotonin and dopamine, are derived from precursors that are found in the diet. Although there are no neurotransmitters directly derived from n-3 PUFAs, variation of dietary intake of these has been shown to influence production of certain neurotransmitters in the brain. Levels of dopamine (Zimmer et al., 2000a), serotonin (de la Presa Owens and Innis, 1999) and acetylcholine (Aid et al., 2003) have been observed to either increase or decrease following either an n-3 enriched or deficient diet, thus potentially having an effect on overall brain function. Indeed, altered levels of neurotransmitters have been implicated in numerous conditions including depression (serotonin - Graeff et al.,

1996), schizophrenia (dopamine - Ohara, 2007), Alzheimer's disease (acetylcholine - Bartus, 2000) and ADHD (dopamine - Nieoullon, 2002), to name a few. Not only is intake of n-3 PUFAs associated with neurotransmitter production, in the frontal cortex dietary supplementation elevated the binding of dopamine at D2 receptors (Chalon et al., 2001), and n-3 PUFA deficiency has been shown to alter dopamine neurotransmission in the nucleus accumbens (Zimmer et al., 2000a). These alterations of the dopaminergic system have been implicated in the reduced learning ability of rats. In addition, abnormalities in animal behaviour following an n-3 deficient diet have also been related to changes in hippocampal acetylcholine (Aid et al., 2003). Further, as previously mentioned, DHA has been shown to modulate ion channel function resulting in a shift in the inactivation curve to a more hyperpolarised potential in the sodium and calcium currents, which could also impact upon neurotransmission (Vreugdenhil et al., 1996). Taken together, these studies suggest that a number of different neurotransmission pathways are susceptible to modification via dietary intake of n-3 PUFAs, which in turn may impact upon behavioural outcomes.

1.4.5 N-3 PUFAs are neuroprotective

In addition to the neuroprotective properties ascribed to DHA as regards its docosanoid derivatives, DHA has also been shown to prevent apoptosis and reduce oxidative stress. Apoptosis, or programmed cell death, is a part of normal, healthy functioning of all multi-cellular organisms, helping to maintain homeostasis. Evidence suggests that specific incorporation of DHA in the phospholipid species phosphatidylserine (PS) in neuronal cell membranes prevents apoptosis. Application of unesterified DHA promotes PS biosynthesis (Hamilton et al., 2000), high levels of which in turn stimulate an increase in activity of a number of enzymes involved in the prevention of this process (Akbar et al., 2005). DHA enrichment of PS only prevents apoptosis in the presence of adverse conditions *in vitro* when cultured cells have been pre-treated with DHA, suggesting that the benefit is only incurred once DHA is metabolised into PS (Kim et al., 2000). Interestingly, DHA appears to encourage apoptosis in non-neuronal cells by down-regulating the expression of antiapoptotic genes and up-regulating several proapapototic ones, implicating DHA in the slowing of proliferation of cancer cells (Narayanan et al.,

2001). Overall it is appears that the antiapoptotic effects of DHA-rich PS in neurons is specific and may be critical for their long-term survival.

Oxidative free radicals are generated under normal physiological conditions but when these molecules are generated in excess, negative consequences may occur. Oxidative cell damage has been linked to a number of conditions including Alzheimer's disease (Markesbery, 1997), ADHD (Selek et al., 2008), depression (Das, 2008) and schizophrenia (Ranjekar et al., 2003). Dietary n-3 PUFAs may play a vital role in oxidative pathology by replacing the lost PUFAs following attack by oxyradicals. Evidence in support of this possibility comes from studies in humans that have shown that oxidant stress was decreased following dietary supplementation with either EPA and DHA or a daily fish meal (Mori et al., 2000). An increase in the activity of two enzymes responsible for removing oxyradicals—xanthine oxidase and superoxide dismutase—has also been demonstrated following an n-3 supplemented diet, providing an alternative explanation (Songur et al., 2004). Oxidative damage following a traumatic brain injury was also reduced in rats fed n-3 PUFAs prior to the injury and also attenuated reduced learning on the Morris Water Maze task compared to controls, providing further evidence of the role of n-3 PUFAs in reducing oxidative stress. One last point to note is that high doses of n-3 PUFAs might actually be a source of oxidative stress; 5.3 g/d DHA+EPA increased lipid peroxidation in healthy adult men, even in the presence of vitamin E (Allard et al., 1997).

1.4.6 The cardiovascular effects of n-3 PUFAs

Over 30 years ago Bang and colleagues (1976) published a seminal paper attributing the low rate of mortality from ischemic heart disease of the Greenland Inuit to a diet rich in n-3 PUFAs as a result of their high consumption of fish, seal and whale, despite the high total fat content of this diet. Since this publication, further epidemiological studies have found an inverse relationship between fish consumption and cardiovascular disease (e.g Kromhout et al., 1995, Gillum et al., 2000, Rissanen et al., 2000), though one systematic review in the area failed to find any conclusive evidence to suggest reduced risk of cardiovascular events or mortality following treatment with n-3 PUFAs (Hooper et al., 2006). On the other hand, a more recent review concluded that fish oil supplementation was associated

with reduced risk of death from cardiac events but no effect on prevention of arrhythmias or all cause mortality (Leon et al., 2008). Regardless of the inconsistencies in the literature regarding the effects of n-3 PUFAs in preventing cardiovascular disease, there are a number of known beneficial effects of n-3 PUFAs in the cardiovascular system that may contribute to overall cardiovascular health, and their increased intake is currently recommended by the UK government (SACN/COT, 2004), the American Heart Association (Lichtenstein et al., 2006) and international scientific organisations (e.g. ISSFAL, 2004). A number of different cardiovascular parameters have been shown to be modified by dietary n-3 PUFAs including increased arrhythmic threshold via modulation of sodium and calcium ion channels (Kang and Leaf, 1996), decreased platelet aggregation (Mori et al., 1997), lowered triglycerides (Nestel, 2000), lowered blood pressure (Morris et al., 1993, Geleijnse et al., 2002) and improved arterial and endothelial function via increased nitric oxide synthesis (Harris et al., 1997, Armah et al., 2008). Given that cerebrovascular events are a risk factor for neurodegeneration along with the fact that the cardiovascular system is responsible for the delivery of nutrients to the brain, it follows that any compound that modulates cardiovascular parameters could exert a secondary effect on brain function and behaviour.

1.4.7 Summary of the functions of n-3 PUFAs

DHA and EPA are involved in a number of fundamental functions at the cellular level. In the brain, DHA is heavily enriched in the cerebral cortex where its incorporation into the phospholipid bilayer of neural cell membranes confers optimal membrane fluidity resulting in improved membrane function as regards signal transduction and neurotransmission. Further, there is evidence to suggest that the expression of a number of genes and the production of various neurotransmitters is sensitive to dietary intake of n-3 PUFAs, suggesting a role for n-3 PUFAs in these processes. In addition, the DHA and EPA incorporated into cell membranes throughout the body can be subsequently released and metabolised further to produce potent secondary signalling molecules that are essential in the resolution of the immune response and may also be neuroprotective. Finally, dietary n-3 PUFAs modulate a number of cardiovascular parameters, which may contribute to reduced risk of cardiovascular events. Given the fundamental nature of n-3 PUFAs and DHA and EPA in particular, it is plausible that alterations in dietary intake could potentially

impact upon brain function and behaviour. The following section reviews the current literature on the behavioural effects of n-3 PUFAs in animals and humans.

1.5 The behavioural effects of n-3 PUFAs

1.5.1 Animal evidence

Our knowledge of the impact dietary n-3 PUFAs have upon cognitive function has been greatly extended by the investigation of their effects in animals, the majority of which have been conducted using rodents. In these animals, the role of n-3 PUFAs is studied using two methods. The first involves the complete removal of n-3 from the maternal diet (animals are fed chow containing LA but no ALA) throughout gestation and lactation resulting in a 50-80% reduction of DHA in the nervous system of the offspring (Fedorova and Salem, 2006). The second method involves dietary supplementation with n-3 PUFAs either to the mother during gestation or directly to the offspring after birth or weaning. In order to assess the effects of these dietary interventions, a variety of tasks have been used, some of which are described below.

A large number of studies have investigated the relationship between brain DHA concentrations and performance on the Morris Water Maze (MWM) task, a task developed to evaluate spatial learning and memory (Morris, 1984). Reduced latency to find the hidden platform in a pool of water upon which the animal can escape is indicative of better learning on this spatial task, performance on which was positively associated with brain DHA status (Fedorova and Salem, 2006). Moriguchi and Salem (2000) demonstrated that second and third generation n-3 deficient rats had longer escape latencies and delayed acquisition of the task compared to controls. In addition, third generation rats (87% reduction in brain DHA) were found to perform worse than second generation rats (83% reduction in brain DHA). Interestingly, in both sets of animals performance was inversely related to levels of docosapentaenoic acid (DPA, n-6; though another form of DPA is also metabolised from ALA in the n-3 metabolic pathway) in the frontal cortex, suggesting that the reciprocal replacement of DHA with DPA has significant consequences. A 60% reduction in brain DHA is sufficient to reduce performance on this task which cannot be attributed to an overall reduction in 22 carbon fatty acids (both DHA and DPA contain 22 carbon atoms), as animals reared on DPA perform the same as those

solely fed LA (Lim et al., 2005), leading to the conclusion that optimal brain function requires incredible structural specificity of neural phospholipids. Indeed, decrements in spatial task performance can be reversed upon administration of a repletion diet containing both ALA and DHA in third generation n-3 deficient rats which is comparable to the performance of rats fed an n-3 adequate diet, demonstrating further the necessity of n-3 PUFAs on certain behavioural outcomes (Moriguchi and Salem, 2003). In terms of the brain regions that are affected by diets lacking n-3 PUFAs, one group discovered that deficient rats suffered reductions in brain DHA in the range of 39-63% in all seven brain regions that were analysed including the cerebellum, medulla, hypothalamus and midbrain with the greatest reductions observed in the striatum, hippocampus and cortex (Xiao et al., 2006).

Olfactory discrimination tasks have also been used by researchers to investigate the role of n-3 PUFAs in cognitive function in rodents. The advantage of this approach is that it rules out the possibility of performance being adversely affected by impairments to the structural visual system brought about by n-3 PUFA deficiency, a phenomenon that as has previously been demonstrated (Neuringer and Connor, 1986). Two studies established that n-3 deficient animals are capable of learning a strategy to solve two-odour discrimination tasks, but that they repeatedly make more false alarms than their n-3 adequate counterparts (Greiner et al., 1999, Greiner et al., 2001). The authors are quick to highlight however that although these results could indicate poorer learning and memory in the deficient animals, these results could also indicate that the deficient animals have lower attention to the stimulus (due to imbalances in the dopaminergic system) and that further studies are needed to tease out these subtleties.

Other paradigms used to evaluate the behavioural effects of n-3 PUFAs include the radial arm maze (requiring reference memory and working memory) and avoidance (learning) tasks. However, the results from these studies are mixed, with some studies suggesting a benefit of n-3 supplemented diets over n-3 deficient diets while others do not, although methodological oversights may offer one explanation as to why these disparities have been found (reviewed in Fedorova and Salem, 2006). Overall the evidence from these studies indicates that carefully controlled n-3 deficient diets lead to a decrease in levels of brain DHA which is associated with poorer performance on a selection of learning and memory tasks, possibly due to

disruptions in dopaminergic neurotransmission (Delion et al., 1994, Zimmer et al., 2002).

In older rats, impairments in tasks that involve complex motor skills and spatial memory decline throughout the lifespan (Shukitt-Hale et al., 1998), which may be attributable to the observed reductions in brain lipids have been consistently observed in aged animals (e.g. Ulmann et al., 2001). Long-term potentiation (LTP), commonly thought to be the biological process underlying learning and memory, is reduced in aged rats (Landfield et al., 1978). In addition, both AA and DHA are significantly decreased in these animals (McGahon et al., 1999). Interestingly, the ability of rat hippocampal dentate gyrus cells to sustain LTP is negatively correlated with the concentration of both AA and DHA in these cells, suggesting a link between the prevalence of long-chain PUFAs and learning and memory (McGahon et al., 1999). Eight weeks of n-3 PUFA supplementation (10 mg/d DHA) is sufficient to restore membrane DHA, which is accompanied by a reversal of the deficits in the ability to sustain LTP (McGahon et al., 1999). Other studies have shown that DHA supplementation can restore radial arm maze task performance in both n-3 deficient (Gamoh et al., 2001) and n-3 adequate (Carrie et al., 2000) aged rats. Together these investigations in aged animals suggest a theoretical basis for and observable benefit of n-3 PUFA supplementation in reducing or reversing age-related impairments.

1.5.2 Human evidence

In humans, n-3 PUFA deficiency to the extent that is observed in animals is extremely rare and only a handful of cases have ever been reported, most commonly as the result of administration of total parenteral nutrition (feeding exclusively via intravenous drip) containing very little or no ALA. Rough, dry skin and hair, excessive thirst and abnormal vision are common features of this type of deficiency; symptoms which can be reversed once ALA is re-introduced to the diet (Holman et al., 1982). N-3 PUFA status can be determined in humans by measuring the concentrations of ALA, DHA and EPA in peripheral tissues such as serum/plasma or erythrocytes. By comparing the n-3 status of healthy normal volunteers to those of various patient groups, it has been revealed that individuals diagnosed with several neurodevelopmental disorders such as ADHD and autism

(Bell et al., 2000, Burgess et al., 2000, Schuchardt et al., 2009), along with a number of psychiatric conditions including depression (Edwards et al., 1998), schizophrenia (Assies et al., 2001) and Alzheimer's disease and dementia (Conquer et al., 2000), have significantly lower levels of n-3 PUFAs. Collectively these findings again suggest that adequate intake and incorporation of n-3 PUFAs is a requirement for normal functioning. The results from studies that have used n-3 supplementation as treatment for symptoms of these conditions have been mixed, however, and further investigation is required. In the next section the role of n-3 PUFAs in a number of neuropsychiatric and developmental conditions is outlined, along with an evaluation of the current evidence of their use in the treatment of these conditions. The section will end with a review of the current knowledge of the effects of n-3 PUFA supplementation on behavioural outcomes in healthy individuals.

1.5.2.1 The role of n-3 PUFAs in neuropsychiatric conditions

1.5.2.1.1 Depression

The WHO estimates that 121 million people suffer from depression worldwide; in the UK it is estimated that at least two thirds of adults will experience depressed mood of sufficient severity to influence their daily activities, with women twice as likely as men to experience a depressive episode (Anderson et al., 2000). There is growing evidence to support a link between depressed mood and dietary n-3 PUFAs. Firstly, fish consumption is inversely related to prevalence of major depression across different countries worldwide (Hibbeln, 1998). For example, in countries such as Japan, Norway and Iceland where intake of fish and dietary n-3 PUFA are high [0.24-0.44% energy, as opposed to 0.10% (UK, USA), 0.08% (Germany)] major, bipolar and postpartum depression are less prevalent than in countries such as the USA and UK where depression is one of the leading causes of disability (Hibbeln et al., 2006). This inverse relationship between fish consumption and depressive symptoms has also been supported by two other population-based cross-sectional studies in Finland (Tanskanen et al., 2001) and New Zealand (Silvers and Scott, 2002) along with two epidemiological studies of older adults aged > 65 years (Bountziouka et al., 2009, Mamalakis et al., 2006a).

In addition to the data using subjective food diaries to assess n-3 PUFA intake, there are other studies that have established a link between depression and n-3 PUFAs using physiological measures. Lower levels of adipose DHA have been reported in mildly depressed healthy adults compared to controls (Tanskanen et al., 2001). Adipose n-3 PUFAs are also negatively related to depression in elderly individuals (Mamalakis et al., 2002) and in adolescents (Mamalakis et al., 2004b). The same is true of erythrocyte membrane levels, where total n-3 PUFAs and DHA are depleted in depressed patients taking medication compared to controls (Edwards et al., 1998, Peet et al., 1998a). Further, the DHA content of the orbitofrontal cortex in patients with major depressive disorder was found to be significantly lower (by 22%) than matched controls (McNamara et al., 2007).

In the face of the mounting epidemiological and peripheral tissue evidence implicating n-3 PUFAs in the pathophysiology of depression (e.g. Edwards et al., 1998, Mamalakis et al., 2006a, Mamalakis et al., 2004a, Peet et al., 1998a, Tanskanen et al., 2001), intervention trials using n-3 PUFAs as a monotherapy or adjunctive treatment in depressive disorders have produced mixed results, though the populations studied, length of treatment regimen (4-12 weeks) and dose (1 - 9.6 g/d DHA + EPA) and formulation (ratio of DHA:EPA) of treatment have varied widely between studies. Similarly, two meta-analyses of the extant literature published around the same time are not in agreement about the efficacy of n-3 PUFAs in the treatment of depressive disorders. The first of these conducted both best and worst-case scenario analyses in a random-effects model for intervention trials carried out in the treatment of major depression and bipolar disorder, including only those studies that used 1g/d doses in the best-case analysis and all doses in all trials for the worst-case analysis. N-3 PUFA supplementation produced a statistical improvement for both analyses, but the authors note high heterogeneity of the results due to the substantially disparate methodologies between studies and recommend caution interpreting these findings (Freeman et al., 2006b). The second paper included studies that have used n-3 PUFAs as an intervention for depression associated with chronic fatigue, post-partum depression, and personality disorder and used fixed-effects analysis. These authors also note large heterogeneity between studies however their results showed little evidence of efficacy of n-3 PUFAs in the treatment of depressed mood, contradicting the findings of the first meta-analysis (Appleton et al., 2006). Among the possible reasons for this discrepancy are the choice of analysis and the inclusion of large (non-significant)

studies that are diverse in both the type of treatment used and population studied (Richardson, 2008). The largest intervention trial to date, however, examined the effects of 12 weeks dietary supplementation with 1.5 g/d DHA+EPA in 190 mild to moderately depressed volunteers, but found no evidence to support the use of n-3 PUFAs in the treatment of depressive symptoms in this population (Rogers et al., 2008), raising the possibility that the benefit of taking n-3 PUFAs for is negligible. Only further large randomised controlled trials (RCTs) will be able to resolve this issue.

1.5.2.1.2 Postpartum depression

Ten-20% of postpartum women are diagnosed with postpartum depression (PPD). This condition is defined in the DSM-IV as a major depressive episode with the onset within 4 weeks of delivery which can potentially have a negative impact upon the child's development (see Ness et al., 2003 for a review). As maternal stores of fatty acids are depleted during pregnancy to ensure an adequate supply for central nervous system development of the growing neonate, some researchers have explored the hypothesis that without sufficient dietary intake of fatty acids, mothers may increase their risk of suffering from PPD (Holman et al., 1991). In rats, it has been observed that an inadequate supply of dietary DHA is enough to result in a 21% decrease in brain DHA in just one reproductive cycle (Levant et al., 2006), but the extent and possible consequences of depletion in humans has yet to be established. In a cross-national study, Hibbeln (2002) discovered that seafood intake and levels of DHA in breast milk were inversely associated with depressive symptoms as measured by the Edinburgh Postnatal Depression Scale (EPDS) in 22 countries worldwide, but another study of 80 new mothers found no relationship between postnatal n-3 fatty acid status and postnatal depression (Browne et al., 2006). In addition, the results from the few intervention trials that have been conducted in this population generally do not support n-3 PUFAs as a treatment of PPD, though large RCTs are still required. In a small open-label trial, supplementation of 2.96 g/d DHA and EPA starting at between 34 and 36 weeks gestation did not prevent PPD in four out of seven participants (Marangell et al., 2004). Freeman and colleagues have conducted two intervention trials in woman who have been diagnosed with depression following birth. The first of these studies was an open-label pilot trial where participants (N = 15) received approx 1.9 g/d

EPA+DHA for 8 weeks (Freeman et al., 2006a). Authors reported a 40.9% decrease in depressive symptoms on the EPDS but in a second randomised dose-ranging study where treatments ranged from 0.5g to 2.8 g/d as adjunctive treatment to supportive psychotherapy, the authors found no difference between groups, with all groups reporting reduced scores on the EPDS and Hamilton Depression Rating Scale (Freeman et al., 2008). Taken together these studies are not conclusive but do warrant further research. First and foremost, future projects would need to adhere to randomised, double-blind and placebo-controlled procedures.

1.5.2.1.3 Bipolar Disorder

Patients with bipolar disorder (BD) fall into one of two categories: BD I is characterised by the occurrence of one or manic episodes interspersed with episodes of depression and BD II is defined by recurrent moderate to severe major depressive episodes as well as lower intensity manic episodes (American Psychiatric Association 2000). Unfortunately, response to treatment in patients with BD is often poor and there is a need for safer and more effective interventions. To this end, the similarities between the effects of mood stabilizers such as lithium and valporate—commonly used in the treatment of BD—and DHA and EPA, on the enzyme protein kinase C (PKC) have lead researchers to consider n-3 PUFAs as an alternative to standard pharmacological treatment for BD. Further, epidemiological studies have revealed a inverse relationship between seafood consumption and lifetime prevalence rates of BDI, BDII and bipolar spectrum disorder (Noaghiul and Hibbeln, 2003). However, evidence from intervention trials is inconclusive, with some published trials reporting a benefit of n-3 PUFAs (Stoll et al., 1999, Frangou et al., 2006, Sagduyu et al., 2005, Osher et al., 2005) whilst others do not (Keck et al., 2006, Marangell et al., 2003). A systematic review of the extant literature in this area concluded that although n-3 PUFAs are well-tolerated by patients with BD and the evidence seems to show an association between n-3 use and symptom reduction, further studies are required in order to confirm their efficacy in the treatment of BD (Turnbull et al., 2008).

1.5.2.1.4 Schizophrenia

Schizophrenia is a mental illness that affects around 0.4-0.6% of the population and is characterised by impairments in the perception of reality, often resulting in social dysfunction (Bhugra, 2005). The popular 'dopamine hypothesis' of schizophrenia proposes that negative symptoms (flat affect) result from reduced activity of the dopamine systems in the prefrontal area, and positive symptoms (delusions and thought disorder) from increased activity of the dopamine systems in the limbic system (Davis et al., 1991). This theory can explain the relationship between dopamine kinetics and the psychiatric symptoms of schizophrenia, but fails to address the cause of the abnormal activities of dopaminergic neurons (Ohara, 2007). Zimmer and colleagues discovered that rats who had been fed an n-3 deficient diet suffered a reduction in the number of presynaptic dopamine vesicles and also that basal dopamine metabolism is increased (Zimmer et al., 2000b, Zimmer et al., 2000a). Dietary n-3 deficiency has also been shown to reduce the number of D2-receptors in the frontal lobe in both rats (Delion et al., 1994) and in piglets (de la Presa Owens and Innis, 1999). It has also be observed that compared to controls, schizophrenia patients have lower levels of plasma n-3 PUFAs (Assies et al., 2001). Therefore, in an attempt to integrate all of the evidence, Ohara (2007) proposed that the n-3 PUFA abnormalities found in schizophrenia stem from the dysfunction of the enzyme phospholipase A_2 (PLA_2). The action of PLA_2 releases membrane fatty acids at the sn-2 position of membrane phospholipids (AA, EPA and DHA), resulting in the generation of free fatty acids which are subsequently metabolised to produce eicosanoids in the case of AA and EPA (Farooqui et al., 1997) or docosanoids in the case of DHA (Tassoni et al., 2008). It follows that increased activation of PLA_2 observed in patients suffering from schizophrenia may cause the excessive depletion of PUFA from the sn-2 position of cell membrane phospholipids in the body and brain. Dopamine concentration, the number of dopamine vesicles and the number of D2 receptors are decreased in the prefrontal presynaptic terminals (resulting in the negative symptoms) and these decreases having a knock-on effect for the limbic dopamine system (resulting in the positive symptoms) (Ohara, 2007).

Despite the apparent plausibility of this integrated theory, a Cochrane review of PUFA supplementation in schizophrenia concluded that data from the six trials that met the inclusion criteria was inconclusive, and the value of treating schizophrenia

with PUFA remains unfounded (Joy et al., 2006). This conclusion was formed largely on the basis that, of the six trials, only one enrolled more than one hundred participants (Peet and Horrobin, 2002a) and in only one study did the intervention period exceed three months (Fenton et al., 2001). Neither of these studies produced compelling evidence to support the use of n-3 in the treatment of schizophrenia. In the first study (Peet et al., 2001), participants were randomly assigned to one of four possible treatment groups; placebo or 1, 2 or 4 g/day ethyl-EPA. The authors also categorised participants into three further groups according to their concurrent medication (typical, new atypical and clozapine). Although improvements were reported on all rating scales by participants in all treatment groups, for those participants whose concurrent medications fell into the typical or new atypical drug category, improvements were also see in the placebo group, rendering differences between groups non-significant. In those participants who were taking clozapine however, there was very little placebo effect and the responses made by participants in the 2 g/day treatment were significantly better following treatment compared to placebo. In a second trial of seventy-five schizophrenic patients conducted by the same group of authors, participants were randomised to either placebo or 3 g/day ethyl-EPA for 16 weeks; all were receiving concurrent medication (Fenton et al., 2001). No differences were found between the active treatment and placebo groups on any of the outcome measures, though the authors are quick to highlight that unique to this study was the fact that participants in previous studies (e.g. Peet et al., 1995, Peet and Mellor, 1998) were younger and had a shorter duration of illness. Only large, longitudinal RCTs will be able to provide sufficient evidence as to whether n-3 PUFAs have a significant positive impact in the treatment of this illness.

1.5.2.1.5 Age-related cognitive decline and dementia

In the UK the population is ageing and living longer than ever before (ONS, 2009). Cognitive function naturally declines with age and has been attributed to a number of factors including reduced synaptic plasticity, decreased membrane fluidity and increased oxidative damage (Willis et al., 2008). In functional terms ageing is associated with decrements in working memory and top-down control in selective attention (de Fockert, 2005). There is growing evidence, however, that various lifestyle factors can either promote or attenuate cognitive ageing. These include

smoking (Swan and Lessov-Schlaggar, 2007), alcohol consumption (Peters et al., 2008), exercise (Colcombe et al., 2003) and diet (Barberger-Gateau et al., 2007, Del Parigi et al., 2006). In particular, one of the dietary factors that has been explored in detail is intake of fatty acids. For example, the Dutch prospective population-based Zutphen Elderly Study identified that LA was positively associated with cognitive decline over a 3 year period (defined as a >2 point drop in Mini Mental State Examination) in 476 men aged 69-89 years (Kalmijn et al., 1997b). A recent re-analysis of the same data was able to identify that in this sample of elderly men, those who did not eat fish observed a 1.2 point decline in MMSE score at the 5-year follow up, as opposed to only a 0.3 point decline in men who reported eating fish (van Gelder et al., 2007). Additionally, a cross-sectional study by the same group identified that oily fish consumption (measured using a FFQ) was significantly associated with a reduced risk of global cognitive function impairment and psychomotor speed in participants of 45-70 years, independent of other confounding factors (e.g. age, sex, education, smoking, alcohol consumption, energy intake) (Kalmijn et al., 2004a). Findings from the Chicago Health and Aging Project (CHAP), conducted in 2560 participants aged 65 years and older over a period of 6 years also discovered that fish intake was associated with a slower rate of cognitive decline at the 6-year follow up. More specifically, among those who consumed one fish meal per week, decline was 10% slower than those who consumed fish less than weekly and 13% slower for those who consumed two or more fish meals per week, adjusted for age, sex, race, education, cognitive activity, physical activity, alcohol consumption, and total energy intake. What the authors could not conclude is whether it was n-3 PUFAs that were the relevant dietary constituent in fish accountable for this finding (Morris et al., 2005). The prospective population-based Etude du Vieillissement Ateriel (EVA) study evaluated fatty acids in erythrocyte membranes and performance on the MMSE in a sample of 246 63-74 year olds (Heude et al., 2003). These authors found that higher proportions of stearic acid (a saturated fatty acid) and total n-6 PUFAs (LA, AA, γ-linolenic acid (GLA), DPAn-6) were associated with greater risk of cognitive decline and that a higher proportion of total n-3 PUFAs (ALA, DHA, EPA, DPAn-3) was associated with a lower risk of cognitive decline over a 4-year period. Similarly, intake of EPA and DHA (estimated via a food frequency questionnaire) was inversely associated with cognitive impairment (MMSE). Finally, higher plasma n-3 PUFA proportions in a sample of 807 healthy participants aged 50-70 years predicted less decline in

sensorimotor speed and complex speed over a 3 year period, though there were no associations between n-3 PUFA proportions and memory, information processing speed or word fluency, although no significant associations were detected at baseline between n-3 status and performance in any of the 5 assessed cognitive domains (Dullemeijer et al., 2007).

It is only now that large-scale prospective randomised intervention trials are currently being conducted to evaluate the effects of fish oil intervention on cognitive function in older adults. The OPAL (Older People And n-3 Long-chain polyunsaturated fatty acids) study is a 24-month placebo-controlled trial which aimed to assess the effects of a daily fish oil supplement containing 0.5 g DHA and 0.2g EPA on cognitive performance on the California Verbal Learning test and other measures of memory and attention in 867 men and women aged 70-79 years (at baseline) (Dangour et al., 2006). Preliminary results from the baseline data suggest that associations between fish consumption and performance are not significant after adjusting for a range of socioeconomic and heath factors, but only the final analysis will be able to reveal the efficacy of the actual treatment (Dangour et al., 2009). In addition, two further intervention trials from which data have yet to be published, the MIDAS (Memory Improvement with Docosahexaenoic Acid Study - Martek, 2006) and the MAPT (Multidomain Alzheimer Preventative Trial - Gillette, 2009) studies will be able to further elucidate the relationship between n-3 PUFA and cognitive function in older adults.

The progression of age-related cognitive decline to cognitive impairment is rising dramatically the world over and currently around 24 million people are affected by dementia, with Alzheimer's disease accounting for around 60% of cases. With the yearly burden of dementia in the UK alone estimated at £17 billion, defining strategies to prevent or delay cognitive impairment in the elderly should be a priority for healthcare (Knapp and Prince, 2007). A person is diagnosed with dementia when: cognitive impairment is greater than that found in normal ageing, affects two or more cognitive domains and also the person's ability to function (American Psychiatric Association 2000). Alzheimer's disease (AD) is the most common form of dementia affecting around 60% of people, the early stages of which is characterised by apathy, anxiety and depression. As the neurodegeneration increases, symptoms progress to delusions and hallucinations, at which point full-time care or institutionalisation are required (McKeith and Cummings, 2005).

Vascular dementia is the second most common form of dementia, with several different subtypes, and is accompanied by a range of different cognitive impairments of the frontal lobe including verbal fluency, set-shifting and abstract thought (Mariani et al., 2007).

A number of observational studies in humans have examined the relationship between intakes of n-3 PUFAs, as measured by various food frequency questionnaires (FFQ), and diagnosis of dementia or AD, but overall the results are conflicting. Barberger-Gateau et al. (2002) found in their analysis of the PAQUID epidemiological study (*N* = 1674 aged 68 years or more) that those participants who consumed fish or seafood at least once a week were at a lower risk of developing dementia, including AD at the 7 year follow-up. However, after adjusting for education level, which was positively correlated with fish intake, the strength of the association diminished somewhat. A publication from the CHAP cohort demonstrated that after a mean follow up of 3.9 years that a higher intake of DHA and weekly fish consumption reduced the risk of AD, though EPA was not associated with a reduced risk (Morris et al., 2003). Conversely, results from the prospective population-based Rotterdam study (*N* = 5395) found no association between n-3 intake and risk for any type of dementia (Engelhart et al., 2002). Similarly, results from the Canadian Study of Health and Aging also do not suggest that an association between total n-3 PUFAs, DHA or EPA and incidence of dementia or AD (Kroger et al., 2009). In addition, the results from two other large-scale studies that initially indicated an inverse association between n-3 PUFAs and incidence of AD and dementia were attenuated once sex, age and education were adjusted for (Huang et al., 2005, Schaefer et al., 2006).

Despite these mixed reports, the biological basis for pursuing research in this area is compelling; n-3 PUFAs possess three properties by which they may protect against the development of dementia which include increasing cerebral blood flow, attenuating inflammation and reducing amyloid production (reviewed in Fotuhi et al., 2009). Results from animal studies are indeed encouraging; in their review of the protective effects of n-3 PUFAs in Alzheimer's disease, Boudrault et al. (2009) conclude that treatment with DHA in rodent models of AD consistently protects against AD, with a number of observable effects in the brains of animals fed DHA compared to controls including decreased pro-apoptotic proteins and secretion of amyloid beta (Aβ) and increased activity in the PI-3 kinase cascade, a

neuroprotective pathway shown to be reduced in AD. Coupled with these physiological changes are studies showing improvements in cognitive function. One group from Japan have focused particularly on this issue, and have consistently shown protective effects of n-3 PUFA administration on spatial learning ability in Aβ infused rats (Hashimoto et al., 2005a, Hashimoto et al., 2002, Hashimoto et al., 2008, Hashimoto et al., 2005b). However, it is worth nothing that the quantity of n-3 PUFAs given to these animals is 2-4 times greater than the current intake in humans (Boudrault et al., 2009). Interestingly, in humans, levels of DHA in the brains of Alzheimer's disease patients does not significantly differ from normals, though levels of stearic acid (frontal and temporal cortex) and AA (temporal cortex) are reduced, and oleic acid is increased (frontal and temporal cortex), indicating some differences in brain fatty acid composition (Fraser et al., 2009). Compared to animal studies, intervention trials in humans however have not been met with the same success. A dose-ranging intervention in 302 participants aged 65 years or older with a MMSE score of >21 found no effect of either dose of fish oil containing either 400 mg or 1800 mg DHA+EPA on cognitive function (memory, sensorimotor speed, attention, executive function) compared with placebo following 26 weeks of dietary supplementation (van de Rest et al., 2008). Similarly, the OmegaAD clinical trial examined the effects of n-3 PUFA supplementation in 174 patients with mild to moderate AD. In this one-way cross-over trial, the active treatment consisted of daily dietary supplementation with 1.6 g of DHA and 0.6 g EPA. At six months there was no difference between groups on either the MMSE or the Alzheimer's disease Assessment Scale. However, in a subgroup of participants with very mild cognitive dysfunction there was a significant reduction in MMSE decline rate, and this was replicated in the crossover group at 12 months (Freund-Levi et al., 2006). These authors also suggest that in terms of the neuropsychiatric symptoms of AD, carriers of the APOε4 gene might be more susceptible to the effects of treatment with n-3 PUFAs, though this is an avenue of investigation that needs to be pursued further (Freund-Levi et al., 2007). Lim et al. (2006) conclude in their Cochrane review that there is a growing body of evidence from biological, observational and epidemiological studies suggesting a protective effect of n-3 PUFAs against dementia. The level of this effect remains unclear, however, and to date dietary recommendations in relation to fish and n-3 PUFA consumption and risk of dementia cannot be made. It is hoped that the results of the DHA in Slowing the Progression of AD study, a prospective 18 month intervention trial in 400

participants aged 50 or older with mild to moderate cognitive impairment, could be used to inform the efficacy of n-3 PUFA in the prevention of dementia (Quinn, 2007).

1.5.2.2 The role of n-3 PUFAs in neurodevelopmental disorders

Richardson and Ross (2000) were among the first researchers to link neurodevelopmental disorders such as ADHD, dyslexia, developmental coordination disorder (DCD) and autism with n-3 PUFA deficiency. These authors noted clinical commonalities between these conditions such as the preponderance of males that were affected, apparent links between allergies and other immune system disorders such as proneness to infections and atopic conditions, abnormalities of mood, arousal and sleep, as well as cognitive impairments in attention and working memory, which suggest disruptions of visual or auditory processing (Richardson, 2006). It had also been observed some twenty-five years previously that individuals with these conditions also shared physical characteristics seen in animals specifically bred on n-3 deficient diets such as excessive thirst, frequent urination, rough, dry hair and skin and follicular keratosis (Colquhoun and Bunday, 1981). Indeed, several studies in children with ADHD have demonstrated that these children have lower blood concentrations of PUFAs, namely AA, DHA and overall concentrations of n-3 PUFAs (Burgess et al., 2000, Stevens et al., 2003, Stevens et al., 1996, Bekaroglu et al., 1996). Given that there is no evidence to suggest that n-3 PUFA intakes are lower in children with ADHD than in healthy children (Ng et al., 2009), the low levels of n-3 PUFAs found in the blood of children with ADHD have been attributed to either inefficient conversion of ALA to EPA and DHA or enhanced metabolism of these fatty acids (Stevens et al., 1995, Burgess et al., 2000). There have been five widely-cited intervention trials investigating the effectiveness of n-3 PUFA treatment on symptoms in children with ADHD and related developmental disorders. These studies have varied in design but interestingly the three experiments that report a positive effect of treatment all used a daily treatment regimen lasting twelve weeks or longer and the treatments themselves originated from fish oil, and therefore contained both DHA and EPA (Richardson and Montgomery, 2005, Richardson and Puri, 2002, Stevens et al., 2003). The study by Voigt et al. (2001) found no effect of 345 mg/d DHA for 16 weeks on a wide range of behavioural and computerised measures of ADHD-related symptoms in 54 children

diagnosed with ADHD and Hamazaki and Hirayama (2004) found no effect of treatment on behavioural symptoms of ADHD with a daily fish oil supplement for 8 weeks, suggesting the possibility that both the composition of the n-3 PUFA treatment and duration of regimen are key factors in ameliorating symptoms of ADHD and related disorders.

Similarly, a relationship between n-3 fatty acid status and autism has also been demonstrated, though intervention trials showing a pronounced benefit of treatment with n-3 PUFAs are lacking. Vancassel et al. (2001) discovered that DHA was decreased by 23% in the plasma phospholipids of autistic children and total fatty acids by 20%. In contrast, a more recent study found that in 16 high-functioning males with autism, DHA and the ratio between total n-3:n-6 PUFAs were increased in plasma phospholipids compared to 22 matched controls, and consequently the authors advised serious caution against treating this condition with n-3 PUFAs (Sliwinski et al., 2006). Despite this, Amminger et al. (2007) published results from a pilot trial wherein they administered seven 7 diagnosed with autistic disorder 7 g/d fish oil for 6 weeks. When compared to matched controls who received a placebo treatment for the same duration, the only significant difference found between groups was on an irritability scale; no differences were found between groups on the social withdrawal, stereotypy, hyperactivity or inappropriate speech measures. The authors are quick to note the small sample size and the relatively short duration of the trial. No adverse effects on behaviour were observed.

N-3 PUFAs have also been linked to dyslexia, and to this end Richardson et al. (2000) examined the associations between the clinical signs of n-3 fatty acid deficiency (excessive thirst, frequent urination, rough, dry hair and skin etc.) and reading ability, spelling and auditory working memory in 97 dyslexic children. The authors detected inverse associations between signs of n-3 deficiency and reading and overall ability, and in boys alone poorer spelling and auditory working memory. This finding was reflected in a study of dyslexic adults who filled out two self report questionnaires; one on signs of fatty acid deficiency and another concerning signs and severity of dyslexia. The authors reported that the signs of fatty acid deficiency were significantly elevated in dyslexic participants and that this reached higher significance in males (Taylor et al., 2000). Cyhlarova et al. (2007) also recently examined the link between fatty acid status and literacy skills in thirty-two dyslexic individuals and twenty matched controls. For both groups, better word reading was

associated with higher total n-3 concentrations though it was only in dyslexic participants that a negative correlation was found between reading performance and the ratio of AA:EPA and with total n-6 concentrations, despite there being no significant differences in membrane fatty acid levels between groups, suggesting that, as in ADHD, the ratio of n-6:n-3 PUFAs or a intrinsic disruption in the metabolism of these fatty acids may be a contributing factor in the aetiology of these conditions. A collection of preliminary studies reported by Stordy (2000) seems to indicate that impairments of the visual system can be improved with a high-DHA supplement in dyslexic participants, although larger RCTs have yet to be carried out investigating the full extent of the efficacy of n-3 PUFAs in the treatment of dyslexia.

1.5.2.3 N-3 PUFA supplementation and behaviour in healthy individuals

1.5.2.3.1 Infant development

The developing foetus requires a supply of both AA and DHA for structural and metabolic functions (Haggarty, 2004). The brain and retina require a high concentration of DHA to function optimally and as such, it is thought that the n-3 PUFA composition of the maternal diet can affect visual and intellectual development (Innis, 1991). DHA is deposited in foetal fat stores in the last 10 weeks of pregnancy in the quantity of around 10 g. If the diet is devoid of preformed DHA in the first two months of life then this store is mobilised and would be largely used up, supporting critical developmental processes (Farquharson et al., 1993). Whilst the level of AA in breast milk has been found to remain constant at about 0.45% of total fatty acids, the level of DHA, on the other hand, varies with the mother's diet from about 0.1-3.8% of total fatty acids. Unlike breast milk, until relatively recently both term and pre-term infant formulas did not contain any n-6 or n-3 PUFAs and it was observed that formula-fed infants have significantly lower levels of DHA in plasma, erythrocytes and brain cortex compared to breast-fed infants, and lower levels of AA in plasma and erythrocytes (Menon and Dhopeshwarkar, 1983). The consequences of these differences in fatty acid status have been assessed using a variety of methods, with sensory and global cognitive functions along with growth and fatty acid status as outcomes. Overall the available evidence suggests that DHA is essential for cognitive development, though there are discrepancies in the literature.

Studies that have investigated the advantage of breastfeeding over formula on cognitive and developmental outcomes have indeed shown an advantage for breastfeeding, even after controlling for various socio-economic factors. Benefits include improved visual acuity (e.g. Birch et al., 1992, Birch et al., 1998), psychomotor development at 4 months (Agostoni et al., 1995), intelligence (Lucas et al., 1992, Kramer et al., 2008) and even later academic achievement (Horwood and Fergusson, 1998). An obvious limitation of these studies is that human milk contains any number of other components that could be responsible for the observed differences between groups. Other studies have therefore looked at supplemented versus unsupplemented formulas as a more reliable comparison. Supplemented formulas have indeed been shown to be effective in successfully raising infant's levels of AA and DHA to that of infants who have been fed human milk, within about 10%. Carlson et al. (1996) were effective in mimicking the levels of AA and DHA in American women's milk, and when the formula contained 0.1% DHA and 0.43% AA, there were no significant differences in plasma levels of AA and DHA between the breast and formula fed groups of infants. Both AA and DHA have to be present in the formula, however, as supplementation with DHA alone has been shown to result in lower levels of AA between 15-40% (Auestad et al., 1997). By altering the levels of the longer chain fatty acids in supplemented formulas and using unsupplemented formulas (usually containing only LA and ALA) as a reference group, any developmental effects of these manipulations can be investigated.

Carlson et al. (1996) found only a transient benefit of a supplemented formula (0.1% DHA + 0.43% AA) over an unsupplemented formula (LA:ALA=22:2.2) on visual acuity which was only present at 2 months but not at 4, 6, 9, and 12 months. In a study using a very similar design and levels of DHA and AA, no advantage was seen in the supplemented group at any testing point (1, 2, 4, 6, 9, and 12 months), although the disparity in results could possibly be due to a different sources of fatty acids i.e. egg phospholipids vs. fish oil, respectively (Auestad et al., 2001). Makrides et al. (1995) on the other hand found that infants fed for 4 months on a supplemented formula (0.36% DHA, 0.58% EPA, 1.52% ALA and 0.27% γ-linolenic acid, n-6) had better transient visual evoked potentials (VEP) at 4 and 7.5 months than the standard 1.6% ALA formula, and the same as the infants fed human milk. Birch et al. (1998) also found that infants given higher levels of DHA in two separate supplemented formulas (0.35% DHA and 0.36% DHA + 0.72% AA) had similar steady-state VEP acuity at 6, 17 and 52 weeks to the infants in the human milk

group, and significantly better than the VEP acuity of the standard formula group (LA:ALA=15:1.5). These results suggest that in terms of visual development, the level of DHA in the diet has to be higher than 0.1% to have a beneficial impact.

This theme is continued as regards the effects of supplemented formulas on cognitive function. Only a handful of studies to date have found a positive impact of added n-PUFAs, and these were with DHA at the levels of 0.35 or 0.36% of total fatty acids (Birch et al., 2000, Drover et al., 2009, Birch et al., 2007). Other studies that have used supplemented formulas where the level of DHA added to the formula was around 0.1% DHA (e.g. Lucas et al., 1999, Auestad et al., 2001, Makrides et al., 2000) have failed to show any differences in cognitive or motor development between infants fed a supplemented formula over the standard one.

Altogether these studies indicate an important role for DHA in both visual and cognitive development, and discrepancies between studies can be attributed to methodological variation. Interestingly, as mentioned above, the level of DHA in American mother's milk is estimated at around 0.13% DHA, whereas only higher levels of DHA in the formula have been shown to be effective at producing improvements over placebo in these studies. It is a logical progression to then investigate the developmental impact of supplementing the maternal diet with DHA and other n-3 PUFAs (in the absence of a similar n-6 PUFA shortage in the maternal diet). Several such studies have been conducted with some positive results. Helland et al. (2003) recruited 341 women at 17-19 weeks of their pregnancy and randomly allocated them to a daily regimen of 10 mL of either corn or cod liver oil (1180 mg DHA + 803 mg EPA) until three months after delivery. Plasma levels of DHA were significantly higher in both the infants and the mothers of the cod liver arm compared to the placebo group, demonstrating that maternal dietary supplementation with n-3 PUFAs is reflected in a simultaneous increase in plasma lipid levels of the infant. Fish oil supplementation during pregnancy in this way has been shown to have a positive impact on infant development. The same authors assessed these children at 4 years using the Kaufman Assessment Battery for Children (K-ABC) as an outcome for intelligence and achievement. All children had been breastfed to at least 3 months of age. Those whose mothers were in the cod liver oil treatment group scored higher on the Mental Processing Composite of the K-ABC, and in a multiple regression model, maternal intake of DHA was the only variable to significantly predict this difference in mental processing at age 4 (Helland

et al., 2003). In another randomised double-blind trial study, children whose mothers had been given a fish oil supplement (2.2 g DHA + 1.1 g EPA; *N* = 33) had better hand-eye coordination at 2.5 years of age than those who mothers had been given olive oil (*N* = 39) during pregnancy (Dunstan et al., 2008). There were, however, no significant differences between groups on measures of receptive language or behaviour. It is worth noting that maternal supplementation with 2.82 g/d ALA from week 14 of pregnancy to 32 weeks following delivery had no impact on either the infant's DHA status as measured by plasma lipid levels or on their cognitive function compared to the control group suggesting that the infant requires preformed DHA to meet requirements (de Groot et al., 2004).

In a prospective epidemiological study, Hibbeln et al. (2007) investigated mother's seafood consumption during pregnancy. After adjustment for twenty-eight potential confounding factors, these authors reported that consumption of less than 340 g of seafood per week was associated with increased risk for suboptimal outcomes for prosocial behaviour and fine motor, communication and social development scores and increased risk for being the lowest quartile for verbal intelligence. This study is the largest of its kind (*N* = 11,875) and provides strong evidence that maternal fish and fish oil consumption can have an important positive impact on infant development.

1.5.2.3.2 Normally developing children and healthy adults

As noted in the previous section, n-3 PUFAs appear to be important for certain aspects of early development. A logical progression is to therefore examine the behavioural effects of n-3 PUFAs in children and young adults. However, very few intervention studies assessing behavioural parameters in healthy individuals have been conducted. As regards cognitive outcomes, three studies in children (Dalton et al., 2009, Osendarp et al., 2007, Ryan and Nelson, 2008) and four in adults (Fontani et al., 2005, Rogers et al., 2008, Hamazaki et al., 1996, Antypa et al., 2009) have included measures of cognitive function in n-3 PUFA intervention trials.

Dalton et al. (2009) found beneficial effects of a daily fish flour spread given to healthy children aged 7-9 years (*N* = 183) for 6 months in a single-blind placebo-controlled trial. Following the treatment, which contained 335 mg ALA, 82 mg EPA and 192 mg DHA, participant's plasma and erythrocyte concentrations of EPA and

DHA significantly increased in the active treatment group and AA significantly decreased. This was coupled with improvements on the Hopkins Verbal Learning Recognition and Discrimination outcomes (Immediate and Delayed Word Recall, Word Recognition), as well as on a spelling test. Marginally significant results were also seen on a reading test, compared to placebo. Another study in a similar age group did not report benefits of a fish oil intervention, though the quantities of n-3 PUFAs contained in the supplement were much lower (Osendarp et al., 2007). In this study, as part of a large trial investigating micronutrient supplementation in healthy Australian and Indonesian school children aged 6-10 years (*N* = 780), one arm of the study consisted of an n-3 PUFA intervention, containing 88 mg DHA and 22 mg EPA. Despite the intervention successfully increasing plasma n-3 PUFA concentrations, there was no effect of treatment on any of the cognitive outcome measures, which included tasks to measure general intelligence, verbal learning and visual attention. Similarly, another study in a slightly younger age group (4 years, *N* = 175) examined the effect of 400 mg/d algal DHA following 4 months of supplementation and found no effect of treatment on any of the outcomes that included measures of memory, attention, vocabulary acquisition, listening comprehension and impulsivity, though the authors did find evidence of a positive association between blood concentrations of DHA and performance on a measure of vocabulary acquisition and listening comprehension (Ryan and Nelson, 2008).

The four studies conducted in healthy adults that have assessed cognitive performance have also reported mixed results. Fontani et al. (2005) gave participants 4 g fish oil containing 1.60 g EPA and 0.80 g DHA a day for 35 days (*N* = 33, mean age 33 years). They were then tested on several tests of attention prior to and following this regimen of supplementation. Results showed that participants' reaction times were reduced on the Go/No-Go task and Sustained Attention task following supplementation. It is noteworthy, however, that although this study had a parallel group (*n* = 16) that were administered a placebo containing 4 g/d olive oil, and the results for this group were null after the trial period, the results of the two groups were not compared in the analysis and therefore the claims of efficacy of n-3 supplementation in this study should be regarded with caution. In comparison, Antypa et al. (2009) followed a similar protocol to Fontani et al. (2005) where they also looked at the effects of a comparable quantity of n-3 PUFAs (1.75 g EPA+ 0.25 g DHA) administered for 4 weeks on depression-relevant cognition and mood in healthy adults. However, these authors did not detect any effects of treatment on

any of the cognitive measures including attention, memory, response inhibition and emotion recognition. A much larger double-blind placebo-controlled intervention trial in 190 mild-to-moderately depressed but otherwise healthy adults only found a trend for an effect of treatment on an impulsivity task, and no other significant results from any other cognitive measures used to investigate the effects of a daily fish oil supplement containing 630 mg EPA and 850 mg DHA for 12 weeks (Rogers et al., 2008). Lastly, Hamazaki et al. (1996) reported an effect of 1.5-1.8 g/d DHA in preventing an increase in extraggression (aggression towards others) during times of mental stress (university exams) in healthy young adults, but no effect of treatment on a Stroop task (a task of response inhibition) or dementia detecting tests, in keeping with the mixed findings reported in this area.

1.5.3 Summary of the behavioural effects of n-3 PUFAs

Using models of n-3 PUFA deficiency and subsequent repletion, research that has investigated the effects of n-3 PUFAs on behavioural outcomes in animals has demonstrated that brain depletion of n-3 PUFAs occurs in the complete absence of dietary n-3, and is associated with cognitive costs which can be ameliorated once n-3 PUFAs are reintroduced into the diet. Human studies have been far less conclusive. Low n-3 PUFA status is associated with poorer behavioural outcomes, but the evidence provided by intervention studies in the treatment of conditions such as depression, schizophrenia, ADHD and dementia has been mixed and inconclusive as a whole, although results from a few positive studies have been compelling enough to pursue further research in these areas. As regards the latter, conclusive data regarding the efficacy of n-3 PUFAs in preventing cognitive decline and dementia has yet to be published, and results from a number of large prospective longitudinal intervention trials that are currently being conducted are eagerly awaited. In terms of the relationship between dietary n-3 PUFAs and behavioural outcomes in normal healthy individuals, to date bulk of the research has focused on the effects of n-3 PUFAs on infant development, though a limited number of trials have been conducted in older children and young adults. Overall the benefit of providing n-3 PUFAs to infants on behavioural outcomes appears to be transient, though the majority of studies have only evaluated the effects of relatively low amounts of DHA, and those providing more than 0.3% DHA in the formula have been more effective. Longitudinal research suggests that maternal

supplementation with n-3 PUFAs during gestation through to breastfeeding could confer long-term behavioural benefits. The issue of cognitive enhancement via n-3 PUFA supplementation in normal children and healthy young adults has yet to be adequately addressed, but initial research indicates that supplementation may result in improved behavioural outcomes, though the area is lacking in well designed and executed studies.

1.6 Rationale

The breadth of the above evidence regarding the physiological and behavioural effects of n-3 PUFAs is vast. The available evidence suggests that these fatty acids, which are incorporated into every single cell in the body and can only be acquired via dietary sources, have a significant impact on physiology and behaviour when adequate intake is not met. A direct causal relationship has been most clearly demonstrated in animals; although the available evidence suggests that reduced intake and/or tissue incorporation is associated with poorer health outcomes in humans, establishing a causal link between n-3 PUFAs and behaviour has been largely inconclusive. Overall, mixed findings have been published from studies in the same areas possibly in part due to the varied methodologies employed; sample size, and type, dose and duration of the interventions have differed greatly, and as a result firm conclusions about the effects of n-3 PUFAs on behavioural outcomes have been difficult to draw.

One particular population that has been largely overlooked is healthy individuals; very few data exist regarding the effects of dietary n-3 PUFAs on cognitive function and mood in both healthy children and adults. In the UK, as in many other Western countries, intake of n-3 PUFAs is low; the last National Diet and Nutrition Survey revealed that 74% of the adult population aged 19-64 years does not consume any oily fish, the predominant source of the more biologically active long-chain n-3 PUFAs, DHA and EPA (Henderson et al., 2002). Given the above evidence regarding both the fundamental nature of n-3 PUFAs and the adverse correlates of low n-3 PUFA intake, evaluating the effects of n-3 PUFA supplements in otherwise healthy individuals seems both logical and valid. Should supplementation in healthy children and adults confer a benefit on behavioural outcomes, then these results would not only be able to increase our knowledge of the functions of n-3 PUFAs, but

could also be used to inform dietary advice. To this end, Chapters 2, 3 and 4 will investigate the relationship between dietary n-3 PUFAs and cognitive function and mood in healthy children (Chapter 2) and adults (Chapters, 3 and 4), with a view to evaluate their efficacy for cognitive enhancement.

It is also clear from the research presented above that the functions of n-3 PUFAs in the brain have been studied in great detail at the cellular level, but their effects on brain physiology have yet to be investigated in humans. The studies described in Chapters 5 and 6 specifically evaluate the effects of n-3 PUFAs on cerebral hemodynamics, and Chapter 6 examines the effects of n-3 PUFAs on cerebral hemodynamics and cognitive function in parallel, with a view to further unravel the relationship between dietary n-3 PUFAs and behaviour. This thesis will therefore address three general research questions:

- What is the nature of the relationship between dietary n-3 PUFAs and behaviour in healthy individuals?

- Are n-3 PUFA supplements effective as a cognitive enhancer?

- What are the cerebral hemodynamic effects of dietary n-3 PUFAs?

The studies that comprise this thesis include the first investigation of the effects of DHA on specific cognitive functions and mood in school-aged children; further reports of the effects of n-3 PUFAs on cognitive and function in mood in healthy adults using a novel approach comparing the effects of DHA-rich and EPA-rich fish oil, and the first investigation of the effects of n-3 PUFAs on cerebral hemodynamics in humans.

CHAPTER 2. COGNITIVE AND MOOD EFFECTS OF 8 WEEKS' SUPPLEMENTATION WITH 400 MG OR 1000 MG DHA IN HEALTHY CHILDREN AGED 10-12 YEARS

2.1 Introduction

The polyunsaturated fatty acid DHA cannot be synthesised by the body and must be acquired via the diet. Although all cell membranes in the body contain this fatty acid, DHA is heavily concentrated in the membranes of selected tissues, namely synaptosomes, retinal rod outer segments and sperm, indicating a specialised role for DHA in these cells. Incorporation of DHA into the phospholipids of the cell membrane bilayer has been shown to result in changes to some of the basic properties of the membrane including increased membrane fluidity, permeability, fusion and protein activity (see Section 1.4.1). More specifically to neural cell membranes, adequate incorporation of DHA in this tissue has been shown to support a wide range of processes including enzyme activity and ion channel function, neurotransmission and gene expression (see Sections 1.4.1, 1.4.3, 1.4.4).

The DHA found in neural cell membranes amounts to 10-20% of total fatty acid composition of the brain (McNamara and Carlson, 2006), a large proportion of which is rapidly accrued during the third trimester (Clandinin et al., 1980b), although levels have been shown to continually increase up to the age of 18 years (Carver et al., 2001). Given that DHA appears to function at a fundamental level, it follows that inadequate incorporation of DHA during development would have a negative impact upon neurological functioning. Indeed, there is evidence to suggest that deficiencies in dietary DHA can result in a number of peripheral and central nervous system impairments. These include impairments to visual acuity, cognitive development and other neurological functions (McNamara and Carlson, 2006). There is also emerging evidence that lower levels of n-3 PUFAs (as measured by plasma or erythrocyte concentrations) are associated with psychiatric and neurological disorders in adults (Edwards et al., 1998, Assies et al., 2001, Conquer et al., 2000) and a cluster of common, related childhood disorders including attention-deficit/hyperactivity disorder and autism (Bell et al., 2000, Burgess et al., 2000, Schuchardt et al., 2009). However, a number of intervention studies that assessed the behavioural effects of

dietary supplementation with either EPA and/or DHA in children suffering from the neurodevelopmental disorders mentioned above have been met with mixed results, with some studies reporting a beneficial effect of treatment upon symptoms (Richardson and Montgomery, 2005, Richardson and Puri, 2002, Stevens et al., 2003), while others found no effect (Hamazaki and Hirayama, 2004, Voigt et al., 2001), though differences in sample size, treatment formulation, time-on-treatment and behavioural measures varied greatly between studies (see Section 1.5.2.2).

To date, the effects of DHA supplementation on cognitive function in normal healthy volunteers has predominantly focused on supplementing the diets of infants with a DHA-enriched formula. However the results from these trials have been mixed, with less than half of the randomised controlled trials reporting a beneficial effect of supplemented formula on visual, mental and psychomotor functions in full-term infants (Fleith and Clandinin, 2005). At the time of this study's inception, only one intervention trial had evaluated the effects of an n-3 PUFA supplement in healthy children. This study examined the effects of 3.6 g DHA + 0.84 g EPA per week for 3 months on the Picture Frustration task (a measure of aggression), plus the Hostility-Aggression Questionnaire for Children, in 9 to 12 year old children (Itomura et al., 2005). The results from this study were mixed, with the authors loosely concluding that fatty acid nutrition may effect physical aggression in school-aged girls. Despite this lack of empirical evidence, a number of UK schools had begun to trial schemes where free fish oil or omega-3 supplements were given to pupils and their academic achievement was monitored ("Fish oil study's GCSE successes" 2006; "Pupils test fish brain food pills" 2006), motivated by the promising and the much publicised results from the 'Oxford-Durham Trial'. In this study, the behaviour and reading and spelling ability of children diagnosed with Developmental Coordination Disorder receiving n-3 PUFAs for 3-6 months dramatically improved (Richardson and Montgomery, 2005). These subsequent 'bandwagon' trials were hailed as a resounding success, so much so that the Department for Education and Skills commissioned a report with the Food Standards Agency to investigate whether all children should receive omega-3 at school to bolster performance (Oakeshott, 2006). Unfortunately, these studies were poorly conducted, and none adhered to double-blind or placebo-controlled protocols. Consequently, the effect of n-3 PUFA supplementation in an unselected population of cognitively intact children remains to be adequately investigated. The evidence that the putative benefits of n-3 PUFAs will include direct modulation of behavioural parameters is also far from conclusive.

In this double-blind, randomised, placebo-controlled, parallel-groups, dose-ranging study the effects of 8 weeks dietary supplementation with two separate doses of the n-3 PUFA DHA on the cognitive performance and mood of 90 normally-developing healthy children aged between 10 and 12 years were therefore assessed. A comprehensive assessment of cognitive performance and subjective mood was undertaken in the laboratory pre-breakfast, and at 1 hour and 3 hours following a standard breakfast on the day before commencement of treatment and the last day of a treatment regime.

2.2 Materials and Methods

2.2.1 Design

A placebo-controlled, double-blind design was employed with participants randomly assigned to one of three treatment groups (placebo, 400 mg DHA, 1000 mg DHA; see Section 2.2.3). For the primary outcome measures, assessment (pre-breakfast, 3h post-dose; see Section 2.2.4.1) comprised a second, within subjects factor. Prior to the start of the study a restricted randomisation (30 X 3 treatments) list matching treatments to participant code numbers was computer generated. Participants were assigned to the next available participant code and therefore corresponding treatment at the end of their introductory visit to the laboratory.

2.2.2 Participants

Ninety male and female children aged 10 to 12 years, who were rated by themselves and parents as being healthy, took part in the study. Participants were recruited using publicity sent to local newspapers and schools in the Newcastle-upon-Tyne area. They were not admitted to the study if any of the following criteria were present: food allergies or intolerance, use of any prescription, illicit, herbal or recreational drugs including alcohol and tobacco, use of dietary supplements or fish oil within the preceding 3 months, diagnosis with ADHD, any medical disorder that might potentially interfere with the participation in the testing sessions, consumption of significant levels of fish prior to (> twice per week) and during the study period.

Of the total cohort of 90 participants enrolled into the study one participant withdrew (for non study-related reasons). A further participant's data was removed due to failure to take the treatments according to protocol. Eighty-eight participants therefore contributed datasets to the per-protocol analyses (see Table 2.1 for demographics). Due to two data capture errors only 86 participants provided a full set of data for inclusion in the analysis of the Internet Battery assessments (1 hour post-breakfast on Day –1 and Day 56).

Table 2.1. Participant demographic details recorded at Baseline by condition. Means ± SEM given where appropriate.

	Placebo	400 mg DHA	1000 mg DHA
N (M/F)	12/18	17/9	15/15
Age	10.87 0.18	11.11 0.15	10.70 0.15
BMI	18.64 0.50	18.16 0.59	18.02 0.57

Following a blind data review, a small number of individuals' scores on single measures were excluded from the analysis due to failure to perform the task correctly according to pre-defined criteria (e.g. performance on a task that was at chance or consistent with use of a single button). Where the analysis for a specific task has been based on a reduced data-set the total number of participants contributing is reflected in Tables 2.2 and 2.3. The study was approved by the Northumbria University School of Psychology and Sport Science Ethics Committee and was carried out in accordance with the Declaration of Helsinki (1964). All participants and their guardians gave written informed consent prior to their inclusion in the study.

2.2.3 Treatments

The parents of each participant were given 4 bottles of treatment containing soft-gel capsules (100 capsules per bottle). Two bottles were labelled for consumption in the morning and 2 bottles were labelled for consumption in the evening. To avoid any potential negative gastrointestinal effects of the treatment, two capsules were consumed in the morning and three capsules in the evening, all under parental supervision, with the parent signing a diary card twice daily to confirm consumption. Active treatment capsules contained 500 mg DHASCO-S (Martek Biosciences, Maryland, USA) representing 200 mg DHA (+ approx 4 mg EPA) with the remainder being a commercially available vegetable oil. The placebo capsules contained 500 mg vegetable oil. The combination of morning and evening capsules corresponded to a daily dose of: 0 mg DHA (placebo), 400 mg DHA (active capsules taken in the morning), or 1000 mg DHA (active capsules taken both morning and evening).

The organisation, coding and labelling of the bottles containing capsules was undertaken by a disinterested third party who had no other involvement in the study. With the exception of individual code-break envelopes, which were kept by the

safety officer for the trial (all of which remained unopened), the information matching participant to treatment was not available to the research team until after the blind-data review.

Reference to the treatment diary cards, parental reports and returned capsules suggested that the compliance of all of the participants included in the data-analysis was ≥ 80%. There were no serious adverse events related to taking the active treatments. No participant withdrew from the study due to intolerance of their treatment, and in general the study treatments were well tolerated and that minor adverse events during the 8-week treatment period were equally distributed across the placebo and active treatment conditions.

2.2.4 Cognitive and Mood Measures

2.2.4.1 Cognitive Drug Research (CDR) Battery

A tailored version of the Cognitive Drug Research battery (CDR Ltd, Goring-on-Thames, UK) was used. The CDR computerised assessment battery has been used in hundreds of European and North American drug trials, and has been shown to be sensitive to acute cognitive improvements as well as impairments with a wide variety of nutritional substances (e.g. Kennedy et al., 2006, Scholey et al., 1999, Scholey and Kennedy, 2004). In the current study a tailored version of the CDR battery was used that has previously been shown to be sensitive to dietary manipulations in children (Wesnes et al., 2003, Ingwersen et al., 2007).

The selection of computer controlled tasks from the system is administered with parallel forms of the tests being presented at each testing session. Presentation is via VGA colour monitors and, with the exception of written word recall tests, all responses are recorded via two-button (YES/NO) response boxes. All reaction times were recorded in milliseconds. The entire selection of tasks took approximately 20 minutes to complete, administered in the following order:

2.2.4.1.1 Word Presentation

Fifteen words, matched for frequency and concreteness, were presented in sequence on the monitor for the participant to remember. Stimulus duration was 1 second, as was the inter-stimulus interval.

2.2.4.1.2 Immediate Word Recall (short-term secondary memory)

The participant was allowed 60 seconds to write down as many of the words as possible. The task was scored for number correct, and errors.

2.2.4.1.3 Picture Presentation

Twenty photographic images were presented sequentially on the monitor at the rate of 1 every 3 seconds, with a stimulus duration of 1 second, for the participant to remember.

2.2.4.1.4 Simple Reaction Time (psychomotor function/attention)

The participant was instructed to press the 'YES' response button with their preferred hand as quickly as possible every time the word 'YES' was presented on the monitor. Thirty stimuli were presented with an inter-stimulus interval that varied randomly between 1 and 3.5 seconds. Reaction time was recorded.

2.2.4.1.5 Digit Vigilance Task (vigilance/attention)

A target digit was randomly selected and constantly displayed to the right of the monitor screen. A series of digits was presented in the centre of the screen at the rate of 80 per minute and the participant was required to press the 'YES' button as quickly as possible every time the digit in the series matches the target digit. The task lasted three minutes and there were 45 stimulus-target matches. Task measures were accuracy (% correct), reaction time and number of false alarms.

2.2.4.1.6 Choice Reaction Time (selective attention)

Either the word 'NO' or the word 'YES' was presented on the monitor and the participant was required to press the corresponding button as quickly as possible. There were 30 trials, of which the stimulus word was chosen randomly with equal probability, with a randomly varying inter-stimulus interval of between 1 and 3.5 seconds. Reaction time and accuracy (% correct) were recorded.

2.2.4.1.7 Spatial Working Memory

A pictorial representation of a house was presented on the screen with four of its nine windows 'lit up' in white while the other windows were black. The participant was instructed to memorise the position of the illuminated windows. In 36 subsequent presentations of the house, one of the windows was illuminated and the participant decided whether or not this matched one of the illuminated windows in the original presentation. The participant made their response by pressing the 'YES' or 'NO' response button as quickly as possible. Mean reaction time was measured,

and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages.

2.2.4.1.8 Numeric Working Memory

Five digits were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the 'YES' or 'NO' response button as appropriate as quickly as possible. Mean reaction time, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages.

2.2.4.1.9 Delayed Word Recall (secondary memory)

The participant was again given 60 seconds to write down as many of the words presented previously as possible. The task was scored as number correct, and errors.

2.2.4.1.10 Delayed Word Recognition (secondary memory)

The original 15 words plus 15 novel (distractor) words were presented one at a time in a randomised order. For each word the participant indicated whether or not it was included in the original list of words by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction time and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages.

2.2.4.1.11 Delayed Picture Recognition (secondary memory)

The original 20 pictures plus 20 novel (distractor) pictures were presented one at a time in a randomised order. For each picture participants indicated whether or not it was recognised as being from the original series by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages.

2.2.4.2 Internet Battery

The Internet Battery comprised a selection of tasks programmed in JAVA language. Timing of the test battery and reaction times were made independently of the computer's internal timing, guaranteeing consistent presentation of tasks and

accurate timing of responses. The battery has previously been shown to be sensitive to dietary supplementation in children (Haskell et al., 2008). The presentation of parallel versions of stimuli for each individual task was counterbalanced across the assessments. All reaction times were recorded in milliseconds. The battery was completed in approximately 15 minutes.

The battery included the following cognitive performance elements (cognitive domain in brackets where appropriate) and visual analogue mood items:

2.2.4.2.1 Word Presentation

Fifteen words appropriate for the age range of the participants drawn from the Economic and Social Research Council's 'Children's Printed Word Database' (Masterson et al., 2003) and matched for familiarity, concreteness, and frequency were presented at the commencement of the battery. Stimulus duration was one second, as was the inter-stimulus duration.

2.2.4.2.2 Picture Presentation

Twelve age appropriate line drawings of items (from: Snograss and Vanderwart 1980) were presented at the commencement of the battery. Stimulus duration was one second, with a two second inter-stimulus duration.

2.2.4.2.3 Arrow Reaction Time Test (psychomotor performance/attention)

An arrow appeared on the screen pointing to the left or right. Participants responded with a left or right arrow key press corresponding to the direction of the stimulus arrow. Each of the 80 stimuli remained on screen until the key press was registered. There was a randomly varying inter-stimulus interval of between 1 and 3 seconds. Outcomes were accuracy (% correct) and reaction time.

2.2.4.2.4 Arrow Flanker Test (focused/selective attention)

Five symbols appeared on screen, with the centre symbol always being an arrow pointing to the left or right. The task was to press the right or left arrow key corresponding to the direction of the central arrow. The flanking pairs of symbols could be squares, crosses, congruent arrows (pointing in the same direction), or incongruent arrows (pointing in the opposite direction). Each of the 80 stimuli remained on screen until the key press was registered. There was a randomly varying inter-stimulus interval of between 1 and 3 seconds. Outcomes were accuracy (% correct) and reaction time.

2.2.4.2.5 Paired Associate Learning (spatial working memory)

Two shape symbols (e.g. square, circle, triangle etc) were displayed on the screen side by side for three seconds. Each of the two symbols was then repeatedly presented alone in random order in the centre of the screen for a total of 10 repetitions. The participant had to indicate if the symbol was originally seen on the left or right with a corresponding key press. A second pair of symbols was then presented and the four symbols that were contained in the two pairs were repeatedly presented (10 repetitions), with the participant once again indicating whether each symbol was originally presented on the left or right. This was repeated a further two times until responses were being made to one of eight symbols. Outcomes include the number of errors, and reaction time.

2.2.4.2.6 Sentence Verification (semantic memory retrieval)

Fifty short sentences appeared on screen that were either true (e.g. 'Bicycles have wheels') or false (e.g. 'Tomatoes have wings'). Participants responded 'true' or 'false' via a key-press as quickly as possible. Outcomes included accuracy (% correct) and reaction time.

2.2.4.2.7 Delayed Word Recognition (secondary memory)

Word recognition was tested by the presentation of the 15 words presented at the commencement of the battery randomly mixed with 15 novel (distractor) words. Participants responded either 'yes' or 'no' by key press to indicate whether the word had previously been presented. Outcomes included accuracy (% correct), and reaction time.

2.2.4.2.8 Delayed Picture Recognition (secondary memory)

Picture recognition was tested by the representation of the 12 drawings presented at commencement of the battery plus 12 novel (distractor) drawings presented in random order. Participants responded either 'yes' or 'no' by key press to indicate whether the picture had previously been presented. Outcomes included accuracy (% correct) and reaction time.

2.2.4.2.9 Mood and Fatigue Visual Analogue Scales (mood)

After completion of the cognitive tasks, participants were then asked to complete a computer-adapted version of the self-report visual analogue scales ("relaxed", "alert", "jittery", "tired", "tense", "headache", "overall mood" that have been used extensively in previous research assessing dietary manipulations e.g. (Haskell et al.,

2005, Rogers et al., 2003). The scale was completed by using the computer mouse to place a cross on a line that represented a continuum between 'not at all' and 'extremely' for each of the seven mood items and a further three; "mental fatigue" ('not at all' to 'extremely') and the bi-polar items 'Do you normally feel' "happy/sad" and "stressed/calm".

2.2.4 Procedure

2.2.4.1 Primary assessment

Participants attended the laboratory on three separate occasions (Training day, the day before treatment commenced [Day -1] and the last day of treatment [Day 56]). Each visit took place at the weekend and the first two visits (Training and Day -1) were seven days apart. Testing took place in a dedicated testing facility in the Cognition and Communication Research Centre at Northumbria University with participants visually isolated from each other during testing.

The Training day visit comprised: obtaining informed consent; training on the CDR battery, Internet Battery and questionnaire measures; health screening; collection of demographic and breakfast consumption data; and random allocation to treatment condition.

Following the Training day participants attended the laboratory at 8 am on the day before commencement of treatment (Day -1) and the last (Day 56) day of the 8-week treatment regimen. On Day -1, parents were given the bottles of study treatment and the diary cards in which they were instructed to record the intake of their child's capsules along with any general health observations. On both testing days, on arrival participants undertook an initial pre-breakfast assessment comprising the CDR battery. They then consumed a standard breakfast on each occasion, chosen to correspond most closely to their habitually consumed breakfast from a selection provided in the laboratory. One hour after breakfast (9:45 am) participants completed the Internet Battery and a further 2 hours later (11:45 am) undertook a second parallel version CDR assessment (see Figure 2.1).

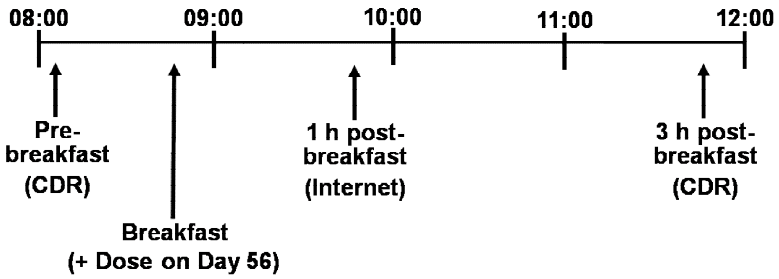

Figure 2.1. Study day running order of assessments. Participants took their final dose of treatment on Day 56 along with their standard breakfast.

The second laboratory visit (Day 56) was identical to the first with the exception that participants consumed their morning dose (2 capsules) immediately following breakfast. Again randomly ordered parallel versions of all tasks were utilised.

Following the completion of testing on Day 56 the participants were asked to return the unused capsules as well as the diary card, and a compliance check (including scrutiny of the compliance diary cards and parental confirmation) was made.

2.2.4.2 Secondary assessment

As part of a secondary, methodological investigation, interim assessments comprising completion of the Internet Battery measures (parallel versions of tasks, and including breakfast consumption, general health, and compliance questionnaire) were undertaken via the Internet in the participant's own home, under parental supervision, on Days 14, 28, and 42. These sessions formed part of an ongoing evaluation of the Internet Battery (e.g. to assess compliance to home testing and test-retest reliability), that was separate to this thesis. A reduced number of full datasets were collected (n = 76); data from these assessments are not included here.

2.2.4.3 Statistical methods

The CDR battery outcomes were analysed by a mixed two-way ANCOVA of the data collected on Day 56 with performance from the appropriate assessment on Day -1 as the covariate. Treatment group (placebo, 400 mg, 1000 mg) comprised the independent measures factor and assessment (pre-breakfast, 3 hours post-

breakfast) formed the within subjects factor. These analyses were conducted using Minitab 15 Statistical Software (Minitab Inc., Pennsylvania, USA). The Internet Battery data were analysed by one-way ANCOVA (treatment group) of the data collected on Day 56, with the data collected on Day -1 as the covariate, and analysed using SPSS statistical software for Windows version 15.0.1 (SPSS Inc., Chicago, IL).

A priori planned comparisons were made between placebo and both the active treatments (400 mg DHA, 1000 mg DHA) utilising t tests with the mean squares error from the ANCOVA as an error term and adjusted means (Keppel, 1991). To ensure the overall protection level against Type I errors, comparisons were strictly planned prior to the commencement of the study and only those comparisons associated with a significant main effect or interaction on the omnibus ANCOVA were calculated.

2.3 Results

2.3.1 CDR battery

Analysis of covariance of data from the pre-breakfast and 3 hours post-breakfast assessments on Day 56 did not reveal any significant effects of treatment or any interactions between condition and assessment. The performance data on this battery for both study days can be found in Table 2.2.

2.3.2 Internet Battery

Analysis of covariance of data on the Internet Battery outcomes on Day 56 (with performance on Day -1 as the covariate) revealed a single main effect of treatment on the visual analogue measure 'alert' [$F(2,82) = 3.88$; $p = 0.025$]. Planned comparisons revealed that ratings of alert were not significantly different from placebo in either the 400 mg condition [$t(82) = 0.89$; $p = 0.375$] or the 1000 mg condition [$t(82) = 1.90$; $p = 0.061$].

Mean performance outcomes from the cognitive measures can be found in Table 2.3 and mean scores on the mood questionnaire items can be found in Table 2.4.

Table 2.2. CDR battery outcomes for placebo, 400 mg and 1000 mg DHA treatment groups on Day -1 and Day 56 of the study at baseline and 3 hours post-breakfast. Means and SEM are presented with *F* and *p* values from the primary ANCOVA.

		Day -1 Assessment					Day 56 Assessment					
			Pre-breakfast		3 h post-breakfast			Pre-dose		3 h post-dose		
		n	Mean	SEM	Mean	SEM	*n*	Mean	SEM	Mean	SEM	*F*	*p*
Immediate Word Recall (no. correct)	Placebo		5.10	0.30	4.50	0.39		4.73	0.31	4.30	0.31		
	400 mg	85	4.73	0.27	4.12	0.32	85	4.77	0.34	3.85	0.35	0.16	0.853
	1000 mg		4.69	0.37	4.52	0.30		4.76	0.37	4.34	0.34		
Simple RT	Placebo		339.04	12.03	387.61	16.28		373.29	14.82	395.79	16.19		
	400 mg	87	352.47	11.33	392.93	17.32	87	389.37	16.64	413.41	19.72	0.54	0.583
	1000 mg		345.82	8.97	410.93	20.32		371.68	10.34	409.59	17.62		
Digit Vigilance (% accuracy)	Placebo		80.79	3.19	75.40	3.45		80.08	2.39	79.05	2.59		
	400 mg	82	74.58	3.37	74.49	3.44	82	76.44	4.06	76.27	3.38	0.21	0.810
	1000 mg		76.78	2.78	73.26	3.20		76.63	2.32	75.86	2.85		
Digit Vigilance (RT)	Placebo		502.40	11.63	534.60	11.68		510.58	9.84	523.30	11.01		
	400 mg		519.27	9.12	532.82	10.49		511.27	9.71	527.23	9.21	0.95	0.389
	1000 mg		504.36	8.34	529.66	10.22		514.84	7.75	531.81	9.47		
Digit Vigilance (false alarms)	Placebo		4.25	0.50	5.39	0.86		3.68	0.47	7.57	2.01		
	400 mg		2.68	0.48	4.88	1.03		2.92	0.46	4.68	0.86	0.90	0.408
	1000 mg		3.79	0.58	5.76	0.83		4.31	0.59	5.79	0.93		
Choice RT (% accuracy)	Placebo		91.11	1.22	89.11	1.39		90.78	1.37	88.67	1.36		
	400 mg	87	93.70	0.95	89.75	1.60	87	91.61	1.17	89.88	1.40	0.17	0.847
	1000 mg		91.00	1.21	90.33	1.26		90.22	1.32	89.00	1.26		
Choice RT	Placebo		552.49	20.04	561.37	19.72		536.78	14.63	595.28	28.21		
	400 mg		547.39	18.54	562.06	22.69		564.80	20.95	571.62	22.65	0.17	0.840
	1000 mg		552.37	16.57	557.30	17.80		555.53	20.23	594.11	21.58		
Spatial Memory (% accuracy)	Placebo		82.76	1.62	89.27	0.90		91.27	2.22	89.40	2.22		
	400 mg	86	85.45	2.04	86.47	1.48	86	89.80	1.79	91.45	1.27	0.01	0.986
	1000 mg		87.50	1.89	88.79	0.79		91.92	1.90	89.72	1.71		
Spatial Memory (RT)	Placebo		884.02	32.38	790.41	37.76		848.09	33.70	791.90	39.71		
	400 mg		948.92	48.91	798.27	48.62		917.23	36.39	775.41	31.46	0.90	0.409
	1000 mg		847.64	33.99	757.22	35.30		862.03	33.26	824.05	43.06		
Numeric Working Memory (% accuracy)	Placebo		83.33	1.64	81.55	1.40		87.33	2.48	88.33	2.26		
	400 mg	88	80.95	1.75	73.81	2.55	88	86.55	2.32	89.29	2.05	0.12	0.890
	1000 mg		84.67	1.41	70.22	2.03		87.33	2.64	88.22	1.60		
Numeric Working Memory(RT)	Placebo		923.44	49.52	933.38	48.90		872.11	43.96	868.36	31.33		
	400 mg		977.83	52.36	900.57	67.37		889.16	46.93	875.15	49.08	0.35	0.703
	1000 mg		894.45	34.39	581.67	28.48		868.07	35.78	884.98	41.51		
Delayed Word Recall (no. correct)	Placebo		3.90	0.29	2.97	0.41		4.03	0.33	3.00	0.31		
	400 mg	86	3.52	0.38	2.15	0.30	86	3.26	0.27	2.19	0.26	2.65	0.074
	1000 mg		3.48	0.34	2.03	0.27		3.83	0.36	2.86	0.41		
Word Recognition (% accuracy)	Placebo		76.11	1.88	65.44	2.12		74.11	2.08	66.56	2.08		
	400 mg	88	74.29	2.66	68.57	2.14	88	74.76	2.01	68.57	2.60	0.65	0.521
	1000 mg		72.67	2.18	64.67	2.24		74.67	1.92	68.11	2.44		
Word Recognition (RT)	Placebo		941.92	32.02	843.45	26.31		893.21	32.11	853.77	36.55		
	400 mg		994.15	49.93	892.77	38.74		907.58	36.76	818.93	37.68	2.79	0.064
	1000 mg		921.60	30.80	846.91	43.94		951.16	41.22	868.09	47.35		

		Day -1 Assessment					Day 56 Assessment					
			Pre-breakfast		3 h post-breakfast			Pre-dose		3 h post-dose		
		n	Mean	SEM	Mean	SEM	*n*	Mean	SEM	Mean	SEM	*F*	*p*
Picture Recognition (% accuracy)	Placebo		85.93	1.60	82.50	2.04		83.58	1.85	78.75	2.02		
	400 mg		82.41	2.04	78.13	1.86		82.14	2.21	76.88	2.77	0.32	0.596
	1000 mg		87.33	1.68	81.67	1.80		84.67	1.45	76.17	2.33		
Picture Recognition (RT)	Placebo		1033.56	26.26	65.00	27.91		953.59	29.49	932.98	32.41		
	400 mg		1068.96	45.06	56.25	34.60		1032.06	46.36	932.28	43.27	2.04	0.133
	1000 mg		1044.80	35.25	63.33	28.33		1030.60	31.35	967.66	38.86		

Table 2.3. Internet Battery performance outcomes for placebo, 400 mg and 1000 mg DHA on Day -1 and Day 56, recorded at 1 hour post-breakfast (Day -1) or 1 hour post dose (Day 56). Means are presented with *F* and *p* values from the primary ANCOVA.

			Day -1		Day 56			
		n	Mean	SEM	Mean	SEM	*F*	*p*
Arrows (RT)	Placebo	86	550.73	22.85	502.93	14.49	2.52	0.086
	400 mg		539.37	17.32	535.00	18.47		
	1000 mg		527.38	22.27	508.93	10.30		
Arrows (% accuracy)	Placebo		92.97	0.93	94.49	0.81	2.83	0.065
	400 mg		92.47	1.14	91.61	1.60		
	1000 mg		93.31	1.25	91.53	1.27		
Arrow Flankers (RT)	Placebo	85	662.72	25.63	632.24	19.84	0.34	0.716
	400 mg		667.26	18.43	644.33	18.34		
	1000 mg		652.48	18.91	641.48	20.93		
Arrow Flankers (% accuracy)	Placebo		94.29	0.95	92.58	1.13	1.66	0.197
	400 mg		95.21	1.13	95.21	1.00		
	1000 mg		93.25	1.45	93.92	0.91		
Paired Associates (RT)	Placebo	85	778.07	31.38	772.97	24.43	1.32	0.274
	400 mg		831.19	36.30	786.81	26.84		
	1000 mg		760.90	27.46	799.83	45.64		
Paired Associates (% accuracy)	Placebo		3.40	0.48	3.67	0.56	0.04	0.960
	400 mg		2.15	0.34	3.19	0.68		
	1000 mg		2.93	0.63	3.59	0.35		
Sentence Verification (RT)	Placebo	85	2400.10	171.39	2012.30	135.85	0.35	0.707
	400 mg		2238.12	107.12	1846.12	95.14		
	1000 mg		2292.62	107.30	1878.31	87.01		
Sentence Verification (% accuracy)	Placebo		85.47	1.78	86.27	2.08	0.23	0.794
	400 mg		88.08	1.74	87.54	2.11		
	1000 mg		89.17	1.59	88.55	1.84		
Picture Recognition (RT)	Placebo	85	844.10	22.99	766.27	21.09	2.07	0.133
	400 mg		854.78	29.35	798.33	25.87		
	1000 mg		871.24	22.34	845.52	30.50		
Picture Recognition (% accuracy)	Placebo		86.59	1.52	84.92	1.81	0.24	0.785
	400 mg		86.08	1.64	83.61	1.78		
	1000 mg		85.68	1.88	82.79	2.16		
Word Recognition (RT)	Placebo	83	845.00	26.36	780.63	38.42	0.71	0.494
	400 mg		889.33	57.17	780.67	24.69		
	1000 mg		865.45	33.99	837.24	49.31		
Word Recognition (% accuracy)	Placebo		78.91	1.74	77.48	1.62	1.18	0.312
	400 mg		79.06	1.47	80.60	1.84		
	1000 mg		77.39	1.43	76.92	1.58		

Table 2.4. Internet Battery mood questionnaire items for placebo, 400 mg and 1000 mg DHA on Day -1 and Day 56, recorded at 1 hour post-breakfast (Day -1) or 1 hour post dose (Day 56). *N* = 86, means and SEM are presented with the F and p values from the primary ANCOVA. All data are in mm.

		Day -1		Day 56			
		Mean	SEM	Mean	SEM	*F*	*p*
Relaxed	Placebo	68.97	2.95	65.13	4.05		
	400 mg	54.74	4.65	68.59	3.90	2.67	0.075
	1000 mg	63.07	4.40	72.72	3.72		
Alert	Placebo	49.50	4.82	56.60	4.90		
	400 mg	57.81	4.14	66.78	3.36	**3.88**	**0.025**
	1000 mg	51.52	4.47	47.10	5.83		
Jittery	Placebo	27.87	4.41	20.63	4.94		
	400 mg	32.74	3.99	28.44	4.17	2.00	0.142
	1000 mg	28.38	4.64	15.48	3.52		
Tired	Placebo	55.57	5.00	42.30	5.25		
	400 mg	61.48	3.64	40.81	4.66	2.95	0.058
	1000 mg	59.24	5.89	53.17	5.55		
Tense	Placebo	22.03	4.01	18.03	4.00		
	400 mg	31.07	3.50	22.19	4.46	0.42	0.657
	1000 mg	26.03	4.09	16.14	3.83		
Headache	Placebo	13.27	4.74	5.67	2.31		
	400 mg	16.07	4.36	14.19	4.34	2.58	0.082
	1000 mg	8.24	2.98	8.14	3.26		
Overall Mood	Placebo	75.73	4.11	80.33	2.83		
	400 mg	72.15	4.17	77.22	3.05	0.57	0.567
	1000 mg	74.52	3.47	82.34	3.46		
Mentally Fatigued	Placebo	37.17	3.28	27.40	4.29		
	400 mg	36.44	4.28	32.56	5.25	1.14	0.326
	1000 mg	37.72	4.87	23.45	4.96		
Happy/Sad	Placebo	14.93	2.81	12.20	2.22		
	400 mg	20.26	2.88	17.67	2.96	0.37	0.690
	1000 mg	17.97	3.08	14.55	3.03		
Stressed/Calm	Placebo	71.53	3.31	78.30	3.77		
	400 mg	66.56	3.85	75.30	3.40	0.03	0.975
	1000 mg	78.17	3.34	80.45	3.36		

2.4 Discussion

The results of the current study found that treatment with either 400 mg or 1000 mg DHA per day for 8 weeks did not have any meaningful effect on the cognitive performance or mood of healthy, 10 to 12 year old children. The entire analysis of data recorded on Day 56 generated a single significant main effect of treatment on participants' self-reported rating of alertness. The planned comparisons, however, failed to detect a difference between ratings from the placebo and either of the active treatment conditions. In addition, the study included a second post-breakfast assessment (3 h) using parallel versions of CDR battery in order to explore any potential effect of the treatments during the natural declines in cognitive performance that have been observed previously (Haskell et al., 2008, Wesnes et al., 2003, Ingwersen et al., 2007), however no treatment x assessment interactions were detected.

The above findings are consistent with other studies that have also not detected an effect of n-3 PUFA supplements on cognitive performance outcomes in similar samples. For example, the report from a large multi-centre randomised trial found no effect of supplementation with 400 mg/d DHA for 4 months in 175 preschool children aged 4 years, although levels of capillary DHA were found to be positively associated with performance on the Peabody Picture Vocabulary Test, a test of listening comprehension and vocabulary acquisition (Ryan and Nelson, 2008). Similarly, another study did not report any benefits of a 12-month fish oil intervention (88 mg DHA + 22 mg EPA) on measures of general intelligence, verbal learning and visual attention in children aged 6-10 years, despite the fact that the intervention successfully increased participants' plasma n-3 PUFA concentrations, compared to placebo (Osendarp et al., 2007). In fact, only one of the three published trials in this population has actually reported a benefit of n-3 PUFAs on cognitive performance outcomes. Dalton et al. (2009) revealed a beneficial effect of a daily fish flour spread (providing 335 mg ALA + 82 mg EPA + 192 mg DHA) on episodic memory (Hopkins Verbal Learning Recognition and Discrimination) and spelling tasks following a 6-month intervention in children aged 7-9 years, compared to placebo. However, given that this study followed a single-blind protocol, along with the null findings reported by the present study and the other published trials described above, does not provide adequate evidence to support n-3 PUFA supplementation for cognitive enhancement in healthy, normally developing children.

Turning back to the evidence provided by the present study, it would be premature to conclude that there is no evidence of efficacy for n-3 PUFAs on cognitive function and mood without first considering several methodological and cohort related factors that might have influenced the pattern of results. The first concerns the length of the treatment intervention. The previous behavioural studies in healthy human participants have adopted treatment regimens of 35 days (Fontani et al., 2005, Antypa et al., 2009) and three months or longer (e.g. Itomura et al., 2005, Dalton et al., 2009, Osendarp et al., 2007). However, given that the results across these studies are less than clear, they provide little guidance on this issue in themselves. Reference to animal models suggests that 8 weeks supplementation with DHA is sufficient to replete brain levels in n-3 PUFA deprived rats (e.g. Moriguchi, Loewke et al. 2001) which has been shown to be accompanied by a simultaneous recovery of behavioural performance (Moriguchi and Salem, 2003). Similarly, cerebral DHA levels in deficient rhesus monkeys were attenuated rapidly, with a doubling of levels within one week, and with total repletion being observed for individuals within 6 to 12 week of commencing supplementation with fish oil (Connor et al., 1990). The evidence provided by these studies suggests that whilst the 8-week treatment regimen employed here would be sufficient for alterations in brain composition to occur, the possibility still remains that this period underestimated the time required for the effects of supplementation to be manifested in behavioural modification.

The nature of the cohort of children is another consideration. The choice of a cross-section of normal, healthy children aged between 10 and 12 years, with a single dietary requirement of low consumption of fish, was made on the basis that this age band is suitable for the type of sophisticated cognitive testing employed and also so that the results could be generalised to other normally developing children, given the lack of data available for this age group at the time of this study's inception. Firstly it should be noted that both the CDR battery (Wesnes et al., 2003, Ingwersen et al., 2007) and the Internet Battery (Haskell et al., 2008) have previously been shown to be sensitive to dietary manipulations in cohorts of a similar age to those assessed here, so the null findings are probably not a result of the cognitive testing employed. Therefore one possibility is that the beneficial effects of DHA on cognitive function, should they exist, might only be seen if supplementation occurs at a putatively more developmentally sensitive period in terms of brain maturation, for instance the last pre-natal trimester or the early postnatal period (Clandinin et al.,

1980b, Clandinin et al., 1980a, Dunstan et al., 2008, Helland et al., 2008, Hibbeln et al., 2007). Considering that the other studies in this area (Dalton et al., 2009, Osendarp et al., 2007, Ryan and Nelson, 2008) have also failed to provide adequate evidence in support of n-3 PUFAs for cognitive enhancement in healthy children, suggests instead that effects of n-3 PUFA supplementation are unlikely to be seen in healthy children that do not exhibit any neurodevelopmental abnormality. It is interesting, however, that the only study to report a benefit of n-3 PUFAs on cognitive function in children also states (from anecdotal reports) that the study population regularly consumed very little to no fish (Dalton et al., 2009), which also raises the possibility that a benefit of n-3 PUFA supplementation may only be observed in children with low n-3 status, a possibility that requires further investigation.

A final consideration is the choice of a predominantly DHA (+ ~2% EPA) supplement. Whilst the beneficial effects of DHA on cell membrane fluidity in the brain may be important, it is also possible that the cellular effects also attributed to EPA or its eicosanoid derivatives, including, for instance, the modulation of ion channels (Jeng et al., 2009), phospholipases (Bell et al., 2004) and gene expression (Salvati et al., 2008) may also be behaviourally relevant. This suggestion is supported by evidence from Ryan and Nelson (2008) who also found no behavioural effects of 400 mg DHA in healthy children, even though the treatment period was longer than the present study, at 3 months. Interestingly, Voigt et al. (2001) and Hamazaki and Hirayama (2004) did not find any effect of DHA administered in isolation on symptoms in children with ADHD, compared with studies that have reported a benefit of n-3 PUFAs who administered a mixture of both DHA and EPA (Richardson and Montgomery, 2005, Richardson and Puri, 2002, Stevens et al., 2003). Even so, the current available data do not provide sufficient evidence to support or refute the choice of either DHA or EPA or even the relative and overall quantity of either n-3 PUFA in investigations of their effects on behaviour. It is hoped that continued research in the area will elucidate this issue further.

In conclusion therefore, the results presented here suggest that there is no evidence of any behavioural benefit of DHA supplementation in a cohort of cognitively intact schoolchildren. This challenges the popularly held, but unsubstantiated, notion that n-3 PUFA supplements will improve brain function and thereby cognitive

performance. The potential for enhancement following similar treatments requires substantial further research.

CHAPTER 3. THE RELATIONSHIP BETWEEN SERUM PUFAs AND COGNITIVE FUNCTION AND MOOD IN HEALTHY ADULTS (18-35 YEARS)

3.1 Introduction

A number of studies have linked reduced dietary intake of n-3 PUFAs and/or low n-3 PUFA status (as indicated by low plasma or erythrocyte concentrations) to a wide range of negative physical and metal health outcomes including but not limited to cardiovascular disease (Mori and Woodman, 2006), immune disorders (Calder, 2007), ADHD and related neurodevelopmental disorders (Richardson, 2006), depression (Freeman, 2006), Alzheimer's disease (Morris et al., 2003) and schizophrenia (Assies et al., 2001). It has been suggested that the rise in incidence of these poor health outcomes is directly related to falling consumption of n-3 PUFA rich oily fish, typical of a 'Western' diet (Hibbeln et al., 2006, Simopoulos, 2000). Much less attention has been given, however, to the study of n-3 PUFAs and specific cognitive functions, and only a handful of studies, all conducted in older adults (60+ years), have examined this relationship. A large population-based study revealed that higher concentrations of n-3 PUFAs in erythrocytes were associated with overall reduced risk of cognitive decline (Heude et al., 2003), and in another study higher baseline plasma n-3 concentrations predicted less decline over 3 years on measures of sensorimotor and complex speed but not memory, information-processing speed or word fluency, though a cross-sectional analysis did not reveal any significant associations between n-3 PUFAs and performance on any of the outcomes at baseline (Dullemeijer et al., 2007). Similarly, estimated intake of DHA+EPA (from a food frequency questionnaire) was inversely associated with information processing speed in participants aged 45-70 years, but no associations were detected with memory, cognitive flexibility or overall cognitive function (Kalmijn et al., 2004a). Another study reported an association between higher proportions of plasma n-3 PUFAs and reduced decline in verbal fluency, but no associations were detected for memory or psychomotor speed (Beydoun et al., 2007). Together these studies suggest that n-3 PUFA status may be associated with reduced risk for cognitive decline in the elderly and that this relationship may be specific to certain tasks rather than global cognitive function, though there is a degree of variation in

the reported findings. Whether cognitive decline can be attenuated via n-3 PUFA supplementation is the aim of a number of currently on-going large-scale research projects (Dangour et al., 2006, Gillette, 2009, Martek, 2006).

As regards evidence provided by supplementation studies, only a limited number of studies in children have measured the relationship between fatty acid status and cognitive function. Dalton et al. (2009) revealed that concentrations of DHA and total n-3 PUFAs in erythrocyte phosphatidylethanolamine were positively associated with performance on a verbal learning task, and the ratio of total n-6:n-3 PUFAs was inversely associated with the same outcomes. No significant associations were detected regarding reading or spelling. Similarly, Ryan and Nelson (2008) revealed that capillary DHA was positively associated with scores on the Peabody Picture Learning Task, though no associations were detected between levels of DHA and performance on any other task (Day-Night Stroop task, Sustained Attention, Continuous Performance Task), again suggesting that peripheral measures of n-3 PUFA status are only associated with certain tasks. To date, no study has examined this relationship in healthy adults, though a limited number of trials have aimed to investigate the effects of n-3 PUFA supplementation on cognitive function. The evidence from intervention trials in healthy adults provides mixed results regarding the efficacy of n-3 PUFAs, with a single study being interpreted as suggesting benefits of supplementation (Fontani et al., 2005), and three others that do not (Rogers et al., 2008, Hamazaki et al., 1996, Antypa et al., 2009), although all these studies varied widely in terms of sample, treatment formulation and duration, and outcome measures.

As regards mood and mood disorders, n-3 PUFAs have also been implicated in their aetiology and/or treatment, with the bulk of research predominantly focusing on depression. In the USA and other developed nations decreased n-3 PUFA intake due to falling oily fish consumption has coincided with increased incidence of depression over the last century (Hibbeln and Salem, 1995) and globally fish consumption is inversely related to the reporting of major depression (Hibbeln, 1998). Similarly, studies that have analysed the fatty acid content of phospholipids in serum/plasma, erythrocyte or adipose tissue have found correlations between lower levels of omega-3 fatty acids and depression in patients (Edwards et al., 1998) as well as community-dwelling elderly individuals (Tiemeier et al., 2003, Mamalakis et al., 2006a) and adolescents (Mamalakis et al., 2006b). There is some

indication that the ratio of AA:EPA may be an important factor with higher ratios being associated with severity of depression (Adams et al., 1996) or the likelihood of developing depression following myocardial infarction (Schins et al., 2007). Lowered DHA and total n-3 PUFAs may also predict the occurrence of post-partum depression (De Vriese et al., 2003) with just a 1% increase in plasma DHA being associated with a 59% reduction in the reporting of depression in mothers (Makrides et al., 2003). However, a significant relationship between n-3 PUFA status and depressed affect has not been universally reported, with one study finding no association between scores on the depression scale of the Depression, Anxiety and Stress Scale (DASS) and plasma concentrations of EPA, DHA or both in combination with the authors suggesting that there is no association between n-3 PUFA status and depressive symptoms in mild to moderately depressed and otherwise healthy adults (Rogers et al., 2008).

Thus it remains unknown whether supplementation with n-3 PUFAs can improve cognitive function and mood in cognitively intact populations, as to date this has only been examined in a limited number of studies. Further to this, it still remains to be established whether a relationship exists between peripheral fatty acid status—known to be reflective of dietary n-3 PUFA intake—and cognitive function in healthy adults. The current study therefore aimed to address this issue by exploring the relationship between serum polyunsaturated fatty acids and specific cognitive performance in an unselected population of healthy adults. To this end, participants completed a range of cognitive tasks evaluating performance across the domains of attention, memory and executive function. Self-report mood assessments were included as secondary measures.

3.2 Materials and Methods

3.2.1 Design

A cross-sectional design was employed. Serum concentrations (% total fatty acid methyl esters, FAME) of DHA, EPA, total n-3 PUFAs (ALA+DHA+EPA; serum concentration data for docosapentaenoic acid were not available) were the independent variables used to assess the relationship between n-3 PUFAs and cognitive function and mood. In addition, as previous research suggests that the ratio of AA:EPA may be important when considering cognitive function (e.g. Fontani et al., 2005, Heude et al., 2003), this relationship along with serum concentrations of AA were also examined.

3.2.2 Participants

Two-hundred and thirty-nine males and females aged 18-35 years were recruited via posters and emails sent to university staff and students. All volunteers were either students attending Northumbria University or were university graduates living in Newcastle-upon-Tyne and the surrounding area. Participants signed a declaration stating that they were in good health and that they were a non-smoker, free from prescription, herbal, illicit or recreational drugs (females taking the contraceptive pill were included) and a native English speaker. Data from participants whose BMI was recorded as 30 or more were excluded from the analysis (n = 20), as it was decided that individuals classed as clinically obese could not be considered in good health. One-hundred and nine participants were also enrolled in the study described in Chapter 4.

The study was approved by the Northumbria University School of Psychology and Sport Science Ethics Committee and was carried out in accordance with the Declaration of Helsinki (1964). All participants gave written informed consent prior to their inclusion in the study.

3.2.3 Assessment of cognitive performance

3.2.3.1 COMPASS

Performance was assessed using the COMPASS (Computerised Mental Performance Assessment) system, a piece of computer software that is used to generate randomised parallel versions of cognitive task batteries. For the present study, COMPASS presented a battery of standard cognitive tasks in the same order as outlined below. All presentations were made via a laptop computer with the exception of the Word Recall and Verbal Fluency tasks, where participants used pen and paper to make their responses. All reaction times were measured in milliseconds.

3.2.3.1.1 Word Presentation

A unique set of fifteen words is presented. Words were selected at random from a large bank of words derived from the MRC Psycholinguistic Database and matched for word length, frequency, familiarity and concreteness. Stimulus duration was one second, as was the inter-stimulus duration.

3.2.3.1.2 Immediate Word Recall (episodic memory)

The participant was allowed 60 seconds to write down as many of the words as possible. The task was scored for number correct, and errors.

3.2.3.1.3 Picture Presentation

Fifteen black-and-white photographic images of objects and outdoor and indoor scenes were presented sequentially on screen for the participant to remember at the rate of 1 every 3 seconds, with a stimulus duration of one second. The same set of fifteen pictures was presented to each participant in a random order.

3.2.3.1.4 Face Presentation

A set of twelve passport-style photographic images of people were presented sequentially in a random order to participants. A first and last name was assigned to each photograph and presented on the screen underneath the person's face. Stimulus duration was one second, with a 3-second inter-stimulus duration.

3.2.3.1.5 Simple Reaction Time (psychomotor performance/attention)

The participant was instructed to press the 'space bar' on the laptop keyboard as quickly as possible every time an upwards pointing arrow appeared on screen. Fifty

stimuli were presented with an inter-stimulus duration that varied randomly between 1 and 3.5 seconds. Mean reaction time was recorded.

3.2.3.1.6 Choice Reaction Time (psychomotor performance/attention)

An arrow appeared on the screen pointing to the left or to the right. Participants responded with a left or right key press corresponding to the direction of the arrow. There was a randomly varying inter-stimulus interval of between 1 and 3 seconds for a total of fifty stimuli. Accuracy (% correct) and mean reaction time were recorded.

3.2.3.1.7 Four Choice Reaction Time (psychomotor performance/attention)

A visual representation of the four direction arrow keys of a standard keyboard was presented on screen. The arrows 'lit up' at random on screen until the corresponding key press was made. In all, each arrow was the target stimulus 12 times, forming a total of 48 stimuli for this task in all. Accuracy and mean reaction time were recorded.

3.2.3.1.8 Stroop Task (attention/response inhibition)

A computerised version of the Stroop task (Stroop, 1992) was created. Words describing colours (GREEN, BLUE, RED, YELLOW) were randomly presented in incongruently coloured text (e.g. GREEN was presented in blue text etc.). For each of the fifty stimulus presentations, participants were instructed to use the computer mouse and cursor to click the colour box located on the right side of the screen that matched the colour of the text the word was presented in. Accuracy and mean reaction time were recorded.

3.2.3.1.9 Verbal Fluency(executive function/semantic memory)

Participants were presented with a letter on screen and were given 60 seconds to write down as many words as they could beginning with that letter. There were 3 separate trials; the letters 'F', 'A' and 'S' were the targets. An overall 'Verbal Fluency' score is achieved by summing the number of permitted words given in each trial.

3.2.3.1.10 Numeric Working Memory

Five random digits from 1-9 were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits (15 targets and 15 distractors) for each of which the participant indicated whether or not it had been in

the original series by a simple key press. The task consisted of 3 separate trials. Accuracy and mean reaction time were recorded.

3.2.3.1.11 Alphabetic Working Memory

This task was identical to Numeric Working Memory, the only difference being that the stimuli were letters.

3.2.3.1.12 Corsi Blocks Task (spatial working memory)

In this task nine identical blue squares appeared on screen in non-overlapping random positions. A set number of blocks changed colour from blue to red in a randomly generated sequence. The cursor was locked in position until the entire sequence had been presented, at which point the participants were instructed to repeat the sequence by clicking on the blocks using the mouse and cursor. The task was repeated five times at each level of difficulty. The sequence span increased from 4 upwards, until the participant can no longer correctly recall the sequence, resulting in a span measure of nonverbal working memory, calculated by averaging the level of the last five correctly completed trials.

3.2.3.1.13 3-back Task (working memory)

A continuous string of letters (upper and lower case; inter-stimulus interval of 2.5 seconds) was presented; 45 letters in total with 15 target pairs. For each stimulus, participants were instructed to indicate whether this was the same letter that appeared three letters before. Accuracy and mean reaction time were recorded.

3.2.3.1.14 Telephone Number Working Memory Task

A nine digit 'telephone number' was presented on screen for 5 seconds which participants were instructed to hold in their memory. After a delay of 10 seconds, participants had to input the number using the numerical numbers at the top of the computer keyboard. Participants scored correctly only if the entire 9 digit number was entered exactly as the number that was presented. There were 8 trials in total. Accuracy and mean total time taken to complete the trials were recorded.

3.2.3.1.15 Delayed Word Recall (episodic memory)

The participant was again given 60 seconds to write down as many of the words presented previously as possible. Total number of correct responses was recorded.

3.2.3.1.16 Delayed Word Recognition (episodic memory)

The original 15 words plus 15 distractor words were presented one at a time in a random order. For each word the participant indicated whether or not it was included in the original list of words by pressing appropriate 'yes' and 'no' keys as quickly as possible. Stimuli remained on screen until an appropriate response had been made. Accuracy and mean reaction time were recorded.

3.2.3.1.17 Delayed Picture Recognition (episodic memory)

The original 15 pictures plus 15 distractor pictures were presented one at a time in a randomised order. For each picture participants indicated whether or not it was recognised as being from the original series by pressing appropriate 'yes' and 'no' keys as quickly as possible. Stimuli remained on screen until an appropriate response had been made. Accuracy and mean reaction time were recorded.

3.2.3.1.18 Names-to-Faces Recall (episodic memory)

The twelve original photographs presented at the outset were again presented on the screen, one at a time. Underneath each picture there was a list of 4 different first names and 4 different last names. For each photograph, participants were instructed to choose the first and last name that was originally presented with the photograph. The numbers of correct responses for first and last names were recorded and collapsed to give an overall score for this task.

3.2.3.2 Cognitive Demand Battery (CDB)

Three repetitions of a 10-minute computerised 'Cognitive Demand Battery' comprised the second half of the testing session. The battery has four components: Serial 3 subtractions (2 min), Serial 7 subtractions (2 min), Rapid Visual Information Processing (RVIP—5 min) and a 'mental fatigue' visual analogue scale. For the serial subtraction tasks, participants subtracted either 3 or 7 consecutively from an original randomly generated number between 800 and 999 for the duration of the task. The RVIP task required that participants respond with a key press every time they detected three consecutive odd or even numbers in a sequence of rapidly (100/min) presented single digits (1-9). Previously the battery has been effectively used to investigate the effects of various nutritional interventions on cognitive and mental fatigue during periods of sustained cognitive processing (see Reay et al.,

2006 for a full description). For this study, data from the three repetitions of the battery was averaged for each of the battery's four component parts.

3.2.4 Assessment of mood

Two subjective ratings of mood were taken. A computerised version of the 16 Bond-Lader visual analogue scales was delivered prior to the cognitive demand battery. The results were combined to form 3 mood factors: 'alert', 'calm' and 'content' (Bond and Lader, 1974). Participants also completed the Depression, Anxiety and Stress Scales (DASS). This 42-item self-report questionnaire requires participants to rate how much each statement of negative emotional state applied to them during the past week. This measure is designed to assess both current state and change in state over time (Appendix I, see Lovibond and Lovibond, 1995 for a full description).

3.2.5 Procedure

Participants attended the laboratory on one single occasion commencing at 8:00 am. On the day of testing, participants were in an overnight fasted state, having consumed nothing but water for approximately 12 hours prior to and for the duration of the session. Testing took place in a suite of laboratories with participants visually isolated from each other. On arrival participants completed a short diet and health questionnaire that included questions about estimated intake of oily fish in a month and whether they currently were taking any form of n-3 dietary supplement (Appendix II). In order to familiarise the participants with the directions and format of each performance measure, a 20-minute practice session was completed with shortened versions of each task. One repetition of the CDB was also completed at this time. Data from the practice session were not analysed. After a 5 minute break the formal testing was carried out and was completed within 75 minutes by all participants. Completion of the DASS was followed by collection of demographic data and a single 5mL blood sample. An outline of the assessment schedule can be found in Figure 3.1.

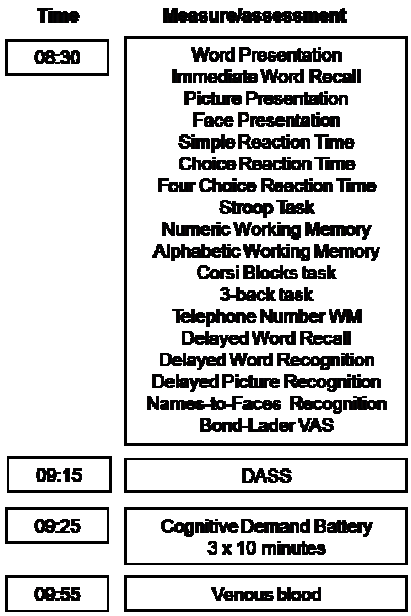

Figure 3.1. Assessment schedule of all tasks and measures. Times are approximate.

3.2.5.1 Blood samples

Blood samples were collected by venipuncture into a serum gel monovette (4.9ml). The samples were immediately centrifuged at 3000 x *g* for 10 min at 20 ºC using an Allegra X-22 centrifuge. The serum was decanted into a 1.5mL microtube and immediately frozen at -80 ºC prior to analysis which took place between December 2007 and January 2009.

Methylation and extraction of the serum fatty acids was carried out using a modified version of the method described by (Masood et al., 2005). The procedure comprised of adding the following components, in order, to a screw-top glass tube: 0.1ml of methyltricosanoate internal standard (Fluka), 0.1ml blood serum sample and 1.8ml of an acetyl chloride / methanol mix (1:25 ratio, prepared immediately prior to the start of the analysis; the methanol contained 50mg/l butylated hydroxytoluene to prevent fatty acid oxidation). The mixture was swirled to mix and then heated to 100°C for sixty minutes. After cooling, 2ml of n-hexane was added to the tube, vigorously mixed for 30 seconds and the n-hexane layer, containing the derivatised fatty acid methyl esters (FAMEs), then transferred to a 2ml micro reaction vessel (Supelco) using a Pasteur pipette. A further 1ml of n-hexane was added to the tube and the process repeated. The n-hexane in the vials was then evaporated to

dryness under a nitrogen flow at 40°C, and the residue re-suspended in 0.1ml n-hexane.

Analysis was carried out using a Thermo Scientific Focus gas chromatograph fitted with a 30m DB5 column (0.25mm internal diameter) and a DSQ mass spectral detector operating in single ion monitoring (SIM) mode to maximise sensitivity (GC conditions were: 140°C initial, hold for 5 minutes, ramp to 280°C at 4°C per minute, hold for two minutes). All components had eluted by 35 minutes. The split ratio was 7:1 and the injection port temperature 270°C. Individual FAME concentrations were calculated from the ratio of peak areas for the component and the internal standard, corrected for the previously determined relative response factor of the component and expressed as a weight percent (i.e. mg per 100mg) of the total FAME concentration (excluding the internal standard).

3.2.5.1 Statistical methods

Associations between performance on the cognitive tasks and mood scales and DHA, EPA, total n-3 PUFAs, AA and the ratio of AA:EPA were explored using simple linear regression and performed with the SPSS statistical software for Windows version 15.0.1 (SPSS Inc., Chicago, IL). All regression analyses were carried out using the relevant fatty acid or ratio as the predictor variable.

Prior to receiving the fatty acid data, scores on individual tasks were excluded if it was evident that the participant had not understood or engaged with the task correctly (e.g. performance on a task that was at chance or consistent with use of a single button, coupled with large variations in reaction times suggesting inattention to the stimuli). This led to participants' scores being excluded in the analysis of the Stoop task (n = 5), Numeric Working Memory task (n = 1) and the RVIP task (n = 11). In addition, three participant's data were missing from the analysis of the Alphabetic Working Memory task and four participants' data for all three repetitions of the CDB due to data capture errors.

An outlier was detected for one participant on the AA:EPA variable, who had a ratio of 115.54 AA:EPA. Cook's distance was utilised to asses the influence of this case on the model for each of the separate analyses. For the majority of regression models Cook's distance ≥ 1, and so the case was removed for these analyses.

Given the large amount of data generated by these analyses, only significant findings are reported in the tables and text in the current chapter; complete analyses can be found in Appendix III.

3.3 Results

3.3.1 Participant characteristics

The mean age of the sample was 22.60 years; 80% were aged between 18-25 years and more females (62%) than males (38%) volunteered to take part. A quarter of participants reported eating oily fish four or more times a month but 26% said they never ate any oily fish. Fourteen participants reported taking an omega-3 supplement, 7 participants took the supplement every day. Self-reported oily fish intake correlated with serum concentrations of DHA [$r(215) = 0.16$, $p = 0.016$] and inversely with AA [$r(216) = -0.17$, $p = 0.010$] but surprisingly not with EPA [$r(214) = -0.05$ NS]. Participant PUFA profiles and demographics can be found in Table 3.1. The levels of DHA in particular are below what has been previously reported in normal healthy adults (e.g. Morse, 2009) though in the current study 63% of participants reported eating 2 portions or less of oily fish a month, which may account for the low observations.

Table 3.1. Participant demographics and serum fatty acid concentrations (% total FAME).

	n	Mean	SEM
Age	219	22.60	0.27
BMI	219	22.84	0.17
Linoleic acid (n-6)	219	33.44	0.30
Arachidonic acid (n-6)	218	6.26	0.15
α-Linolenic acid (n-3)	179	0.24	0.02
Docosahexaenoic acid (n-3)	217	1.04	0.04
Eicosapentaenoic acid (n-3)	216	0.84	0.03
AA:EPA	215	9.49	0.60

3.3.2. Regression analyses

Significant associations between cognitive performance and serum fatty acids are presented in Table 3.2. EPA was found to be positively associated with performance (% accuracy/no. correct) on a cluster of four of the memory tasks; Alphabetic WM, Immediate Word Recall, Picture Recognition, Names-to-Faces Recall. In contrast, DHA was significantly associated with only two outcomes, and in both cases this fatty acid was associated with poorer performance. A negative association was

detected between DHA and accuracy on the Numeric WM task, and positively associated with reaction time on the Telephone Number WM task.

Total n-3 PUFAs were associated with performance in keeping with the above associations relevant to EPA and DHA; total n-3 PUFAs were positively associated with reaction time on the Telephone number WM task, number of correctly recalled items on the Immediate Word Recall task, accuracy on the Picture Recognition task and number of correctly matched names and faces on the Names-to-Faces Recall task.

AA was positively associated with accuracy and number of correct items on the Picture Recognition and Names-to-Faces Recall tasks, respectively. In addition, AA was also positively associated with average reaction time on the RVIP task component of the CDB.

A higher ratio of AA:EPA was predominantly associated with poorer performance; AA:EPA was negatively associated with accuracy on Alphabetic WM and Stroop and number correct on Names-to-Faces Recall. However, AA:EPA was also negatively associated with the Corsi Blocks Speed outcome, indicating faster response time. A positive association was found linking a AA:EPA and mental fatigue and the Anxiety scale of the DASS.

Table 3.2. Means and SEM of performance measures are presented with significant associations between cognitive performance and serum fatty acid concentrations.

Domain	Outcome	Mean	SEM	n^1	Associated Fatty Acid (β; p; 95% C.I.; r^2; n)
Attention	Simple RT	287.70	2.67		
	Choice RT	412.79	3.75		
	Choice RT (% accuracy)	95.75	0.21		
	4 Choice RT	499.52	5.84		
	4 Choice RT (% accuracy)	99.19	0.10		
Working Memory (WM)	Numeric WM (RT)	893.03	15.34		
	Numeric WM (% accuracy)	95.57	0.34		**DHA**(-0.18; 0.010; -2.42, 0.34; 0.03; *216*)[a]
	Alphabetic WM (RT)	547.76	43.09		
	Alphabetic WM (% accuracy)	94.50	0.30		**EPA** (0.16; 0.018; 0.25, 2.57; 0.03; *213*)
					AA:EPA (-0.15; 0.029; -0.33, -0.02; 0.02; *211*)[a]
	Telephone Number WM (RT)	7058.91	199.18		**DHA** (0.15; 0.030; 80.61, 1588.82; 0.02; *217*)
					Total n-3 (0.14; 0.043; 14.82, 860.51; 0.02; *218*)
	Telephone Number WM (% accuracy)	32.48	1.73		
	Corsi blocks Span	5.90	0.08		
	3-Back Task (RT)	1184.81	49.32		
	3-Back Task (% accuracy)	80.89	1.04		
Episodic Memory	Immediate Word Recall (no. correct)	6.21	0.16		**EPA** (0.23; 0.001; 0.049, 1.80; 0.05; *216*)
					Total n-3 (0.17; 0.014; 0.09, 0.77; 0.03; *219*)
					AA:EPA (-0.24; <0.001; -0.18, -0.05; 0.06; *214*)[a]
	Delayed Word Recall (no. correct)	4.73	0.16		
	Word Recognition (RT)	961.92	13.53		
	Word Recognition (% accuracy)	80.35	0.68		

Domain	Outcome	Mean	SEM	n[1]	Associated Fatty Acid (β; p; 95% C.I. .; r^2; n)
	Picture Recognition (RT)	1069.47	16.24		
	Picture Recognition (% accuracy)	87.21	0.77		**EPA** (0.15; 0.027; 0.41, 6.71; 0.02; *216*)
					Total n-3 (0.16; 0.020; 0.30, 3.58; 0.03; *218*)
					AA (0.19; 0.006; 0.29, 1.70; 0.03; *218*)
	Names-to-Faces Recall (no. correct)	10.93	0.40		**EPA** (0.20; 0.003; 0.83, 4.10; 0.04; *216*)
					Total n-3 (0.14; 0.035; 0.07, 1.77; 0.02; *218*)
					AA (0.17; 0.011; 0.11, 0.85; 0.03; *218*)
					AA:EPA (-0.18; 0.008; -0.38, 0.06; 0.03; *214*)[a]
Executive Function	Stroop (RT)	870.36	11.65	214	
	Stroop (% accuracy)	99.45	0.08	214	**AA:EPA** (-0.19; 0.007; -0.08, -0.01; 0.03; *209*)[a]
	Verbal Fluency (total, no. correct)	40.32	0.57		
Cognitive Demand	Serial 3 Subtractions	31.88	0.89	215	
	Serial 7 Subtractions	18.22	0.64		
	RVIP (RT)	477.18	6.39	204	**AA** (0.15; 0.032; 0.53, 12.01; 0.02; *203)*
	RVIP (% accuracy)	45.79	1.41	204	
	RVIP (false alarms)	9.87	0.66	204	
	Mental Fatigue	71.34	1.01	215	**AA:EPA** (0.51; 0.013; 0.11, 0.91; 0.03; *210*)[a]
Mood	Bond-Lader Alert	45.03	0.89		
	Bond-Lader Calm	55.17	0.87		
	Bond-Lader Content	56.75	0.91		
	DASS Stress	10.60	0.51		
	DASS Anxiety	5.10	0.33		**AA:EPA** (0.19; 0.005; 0.03, 0.18; 0.04; *215*) [a]
	DASS Depression	6.84	0.48		

[1] n = 219 unless stated otherwise, [a] Fatty acid/ratio is associated with poorer outcome

3.4 Discussion

Overall, two overarching patterns of results emerged from the individual regression analyses. The first of these was the pattern of positive associations detected between total serum n-3 PUFAs (ALA+DHA+EPA) and performance (% accuracy or no. correct) on a number of episodic memory tasks (Immediate Word Recall, Picture Recognition, Names-to-Faces Recall). It is interesting that several studies in older adults have not reported similar associations between physiological measures of n-3 PUFAs and memory. For example, Dullemeijer et al. (2007), Beydoun et al. (2007) and Kalmijn et al. (2004a) all employed a task that assessed episodic memory (word learning or delayed word recall) in their respective investigations in older adults. However, none of these studies detected an association between individual n-3 PUFAs or total n-3 PUFAs and episodic memory in either cross-sectional or longitudinal analyses. On the other hand, a recent study in children reported associations between DHA, total n-3 PUFAs and the ratio of total n-6/n-3 PUFAs and performance on a task requiring episodic memory following 6 months supplementation with a fish flour spread (Dalton et al., 2009), which, to the author's knowledge, is the only other report of an association between n-3 PUFAs and performance on a memory task. As regards the present study, what is also interesting is that serum concentrations of EPA (and not DHA) were significantly associated with these three same outcome measures, suggesting that it is EPA that is the most relevant component of the total n-3 PUFAs variable. In addition, serum EPA was also positively associated with accuracy on the Alphabetic WM task. Given that there is very little EPA (< 1%) contained in the brain compared to DHA (10-20% total fatty acids - McNamara and Carlson, 2006), these findings were unexpected. However, the many actions of EPA or its eicosanoid derivatives including modulation of ion channels (Jeng et al., 2009), phospholipases (Bell et al., 2004) and gene expression (Salvati et al., 2008) elsewhere in the body may also be behaviourally relevant as regards memory function. This novel finding requires further investigation and an evaluation of the effects of supplementation with EPA in healthy young adults seems justified.

The second pattern that came out the data concerns the ratio of AA:EPA, found to be associated with poorer outcomes in terms of both task performance as well as selected mood measures. Taking the cognitive task performance first, inverse associations were detected between the ratio of AA:EPA and performance (%

accuracy or no. correct) on number of tasks requiring working memory (Alphabetic WM), episodic memory (Immediate Word Recall, Names-to-Faces Recall) and attention/response inhibition (Stroop). An elevated ratio of AA:EPA has been previously associated with a range of adverse health and behaviour outcomes including depression (Adams et al., 1996), reading ability in dyslexics (Cyhlarova et al., 2007) and inflammatory disease (reviewed in Calder, 2006a), but the results of the current study comprise the first report of an inverse relationship between the ratio of AA:EPA and specific cognitive functions. Fontani et al. (2005) reported a significant within-group reduction in the AA:EPA blood fatty acid ratio of participants receiving 4 g/d fish oil for 35 days along with significant within group reductions in participants' reaction time on sustained attention and response inhibition tasks, but unfortunately the authors failed provide data regarding relationship between these outcome measures. Given the above results, consideration of the ratio of AA:EPA in future evaluations of the relationship between PUFAs and cognitive function may be an interesting avenue to pursue.

The ratio of AA:EPA was also positively associated with the Anxiety scale of the DASS as well as with subjective mental fatigue during 30 minutes of cognitively demanding tasks. These findings are particularly interesting in light of the extant literature linking both anxiety and fatigue to inflammatory processes. Epidemiological data suggest a positive association between elevated pro-inflammatory cytokine levels and tiredness (Whalley et al., 2007), and anxiety disorders have also recently been linked to elevated levels of pro-inflammatory cytokines (Hoge et al., 2009). Given that plasma n-3 PUFAs are associated with lower levels of pro-inflammatory cytokines and higher levels of anti-inflammatory ones (Ferrucci et al., 2006), a future extension of this study could investigate the relationship between self-reported mental fatigue and anxiety and these biomarkers, and the effect of increased intake of n-3 PUFAs on both immunological and subjective mental fatigue and anxiety outcomes. It is possible that low-grade inflammation and the ability of n-3 PUFAs to dampen the inflammatory response may link these two findings, though this would need to be confirmed. Interestingly, neither EPA nor AA alone was associated with either of these two mood outcomes, possibly suggesting that the balance between the two PUFAs is more important than individual fatty acid concentrations.

There was no further evidence to suggest a link between mood and n-3 PUFAs in this sample, despite low n-3 PUFA status being previously linked to increased incidence of depression at the epidemiological level (Hibbeln, 1998, Tanskanen et al., 2001, Silvers and Scott, 2002) as well as in clinical studies (Peet et al., 1998b, Edwards et al., 1998, Adams et al., 1996). For this study there were very few participants who scored at the higher end of the DASS (*n* = 15 scoring 21 or more) so it may be that this sample was too homogenous to enable a detection of an association. However, a large intervention trial found that the baseline scores on these same scales were not related to plasma n-3 PUFA concentrations for either high or low scorers (Rogers et al., 2008), in line with the data presented here. Other research does suggest that major depression at least, is associated with a pro-inflammatory phenotype and elevated ratio of n-6:n-3 PUFAs (Dinan et al., 2009), so again, introducing cytokine or other related immunoassays to future intervention study protocols in this area may promote understanding of the subtle relationship between these variables.

Turning to the other individual fatty acids, there was little evidence to suggest that peripheral DHA concentrations are associated with cognitive performance; in fact the two significant associations that were detected revealed that serum DHA is associated with worse performance. This is not consistent with studies in children that have reported a positive association between plasma DHA and measures of learning, reading and spelling (Dalton et al., 2009, Ryan and Nelson, 2008). It is known that the brain relies on an adequate supply of dietary DHA in order to maintain an optimal state of membrane functionality and that DHA also beneficially contributes to a host of processes involved in cellular signalling and neurotransmission (Yehuda et al., 1999, Youdim et al., 2000). However, in piglets at least, concentrations of brain DHA are only related to erythrocyte concentrations of DHA and not plasma concentrations (Blank et al., 2002), suggesting perhaps that there is little justification in associating serum DHA concentrations with cognitive performance. In a similar vein, the detected associations between serum AA and cognitive performance outcomes were also mixed and largely uninterpretable with AA associated with better performance on two of the outcome measures; Corsi Blocks Speed and Names-to-Faces Recall, and with slower reaction time on the RVIP task. AA is essential for optimal brain development and like DHA, is a major structural component of the brain (Innis, 1993). It is not surprising then that erythrocyte concentrations of both AA and DHA have been shown to be associated

with developmental quotient at 24 months (Agostoni et al., 1997). However on the other hand, total n-6 fatty acids have been shown to be associated with greater risk for cognitive decline (Heude et al., 2003). In the case of healthy young adults, given the very limited and mixed findings, the association between AA and cognitive function requires further investigation, though as mentioned above, the balance of AA and EPA may likely be more important than either AA or EPA concentrations alone.

While these results present the possibility that concentrations of individual fatty acids and hence dietary intake are associated with distinct cognitive task performance (memory), overall caution should be exercised when interpreting these associations. Firstly, a large number of regression models were analysed therefore increasing the probability that the significant associations were detected by chance. In light of this, although all the findings have been described, an emphasis has been placed on patterns of results rather than individual associations. Another potential limitation is the possible existence of a covariate(s) that could also account for these findings. The sample was homogenous in terms of age group and education level as all participants were healthy young adults that had attained or were enrolled in an undergraduate level degree. Further, all participants asserted to be non-smokers and medication-free. Other potentially confounding factors such as parental socio-economic status were not controlled for, however, and could be included in future investigations. The use of serum fatty acids as a biomarker of status in this study may have also had an impact on the results. Although widely used as a valid measure of fatty acid intake (e.g. Metherel et al., 2009, Welch et al., 2006), serum fatty acids have a 4-6 times shorter half life (about 1-2 weeks) than those found in erythrocytes (Katan et al., 1997), which are believed to be a more accurate measure of long-term intake and incorporation (Harris and von Schacky, 2004), and therefore potentially a more accurate correlate of cognitive function. In spite of these limitations, what this investigation may have highlighted is that certain cognitive domains are especially sensitive to fluctuations in PUFA status. Indeed, the issue of whether dietary supplementation with n-3 PUFAs has an effect on cognitive performance in cognitively intact adults has yet to be fully investigated.

In summary, it was observed that performance on a number of cognitive tasks is associated with serum n-3 PUFA concentrations; the majority of significant associations indicate a positive relationship between serum n-3 PUFAs and

performance, although this was not universally observed. In addition, a higher ratio of AA:EPA was generally associated with poorer performance as well as increased mental fatigue and anxiety. A causal relationship between n-3 PUFAs and cognitive performance in healthy young adults has yet to be established, however, but given these results further investigation in this population seems justified.

CHAPTER 4. COGNITIVE AND MOOD EFFECTS OF DHA-RICH FISH OIL AND EPA-RICH FISH OIL IN HEALTHY ADULTS (18-35 YEARS)

4.1 Introduction

The connection between nutrition and cognitive function is widely accepted and a large number of varied micro- and macronutrients have been identified as having important actions in the brain that can influence brain function (Fernstrom, 2000). On the one hand, the impact of nutrient deficiency upon behaviour is well documented; deficiencies in Vitamin D (Holick, 2007), folic acid (Reynolds, 2002) and iron (Pollitt, 1993) are all associated with poorer cognitive function and conversely, increasing levels of nutrients via supplementation in otherwise healthy individuals has been shown to have beneficial effects. For example, increased intake of vitamins E and C is associated with reduced cognitive decline in the elderly (Morris et al., 2002, Paleologos et al., 1998, respectively), and multivitamin/mineral supplementation has been shown to have a beneficial effect upon mood and stress in healthy adults (Carroll et al., 2000, Schlebusch et al., 2000, Kennedy et al., In press-a), and measures of attention in children (Haskell et al., 2008). Further, the role of amino acids such as tryptophan and tyrosine, as precursors of neurotransmitters have been explored, with recent evidence suggesting that tryptophan in particular may be able to beneficially modify mood in healthy adult volunteers (Markus et al., 2008).

The behavioural impact of dietary intake of n-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) has also been increasingly examined in recent years. The effect of n-3 PUFA deficiency in animals has been well documented; an inadequate supply of dietary n-3 PUFAs can result in a 50-80% decline in brain DHA and is associated with impaired learning and memory (see Section 1.5.1). Such data are not readily translated into humans, however, where equivalently low levels of n-3 PUFAs are rare, although there is evidence to suggest that lower n-3 PUFA status (compared to controls and measured by plasma/serum or erythrocyte concentrations) is associated with a number of adverse outcomes. These included several neurodevelopmental disorders such as ADHD and autism (Bell et al., 2000, Burgess et al., 2000, Schuchardt et al., 2009), along with a number of psychiatric

conditions including depression (Edwards et al., 1998), schizophrenia (Assies et al., 2001) and Alzheimer's disease and dementia (Conquer et al., 2000). Further to this, the results presented in Chapter 3 suggest that serum PUFA concentrations may be associated with performance on a number of cognitive tasks as well as mood in healthy young adults. Serum concentrations of total n-3 PUFAs (ALA+DHA+EPA) and EPA alone were found to be positively associated with performance on a cluster of episodic memory tasks, and the ratio of AA:EPA was associated with poorer performance on a number of tasks spanning several different cognitive domains (episodic memory, working memory, attention). In addition, AA:EPA was positively associated with subjective anxiety and mental fatigue (see Table 3.2 for details). Together the overall results from the study suggested that dietary supplementation with n-3 PUFAs in this population may be an interesting avenue to pursue, although to date only a handful of studies have addressed this issue. Hamazaki et al. (1996) reported that 1.5-1.8 g/d DHA (from fish oil) for 3 months in healthy students prevented increases in aggression towards others during times of mental stress (university examinations), but did not perform differently than the control group on the Stroop or dementia-detecting tests. Two other studies have examined the effects of dietary supplementation with fish oil on performance and mood. In the first of these Fontani et al. (2005) investigated the effects of 4 g/d fish oil (1.6 g DHA + 0.8 g EPA) for 35 days in healthy amateur athletes. Participants in the fish oil treatment group achieved faster reaction times on a go/no-go task and a sustained attention task, and also improved ratings of anger, anxiety, fatigue, depression, confusion and vigour on the self-report Profile of Mood States (POMS) questionnaire compared to their pre-treatment scores, but no comparison was made between the treatment and placebo groups. Antypa et al. (2009) attempted to directly expand on these findings using a similar sample (healthy adults, but not athletes), intervention period (4 weeks), and comparable quantity of n-3 PUFAs, although the ratio of DHA to EPA was much higher and in the opposite direction (1.75 g EPA+ 0.25 g DHA). These authors specifically aimed to examine the effects of the supplement on depression-relevant cognition and mood, but did not detect any effects of treatment on any of the cognitive measures including attention, memory, response inhibition and emotion recognition; the only reported effect on mood was a small effect on self-rated mental fatigue (POMS). Participants in the n-3 PUFA group did however demonstrate increased risk-seeking behaviour on a gambling decision-making task, which the authors attributed to increased mental

effort and not increased impulsiveness. It must be noted that whilst the statistical analysis of Antypa et al. (2009) was more appropriate than that of Fontani et al. (2005), the authors note two limitations with their study; the effects of the manipulation may have been masked due to the fact that the treatment group had a higher average level of DHA in plasma at baseline and also that levels of DHA inexplicably increased in the placebo group following treatment.

From these studies it is clear the effects of n-3 PUFA supplementation on behavioural outcomes in healthy adults remains to be adequately addressed. Animal studies suggest that the intervention period of both the latter studies would not be sufficient for adequate incorporation of the fatty acids to occur in certain tissues including the brain (Connor and Neuringer, 1988), therefore the reported results may not reflect the effects that might occur following a longer intervention period. Further, a recurrent theme in the n-3 PUFA supplementation literature, both in healthy and patient populations is the use of a wide range of treatment formulations and doses. Both dietary DHA and EPA are known to influence mechanisms that support cognitive function, but given the evidence provided by the studies listed above, it is not possible to say at this time which type of n-3 PUFA supplement, one higher in DHA than EPA ('DHA-rich') or visa versa ('EPA-rich') would have an effect on cognitive performance. In addition, while these aforementioned studies that have specifically examined cognitive function in healthy adults are useful for 'proof of concept', the doses involved would not be easily achieved by dietary intake of oily fish alone, and therefore not readily translated into meaningful advice for the general population. Lastly, the results provided by the study described in Chapter 3 suggest that n-3 PUFA status is associated with a number of cognitive functions in healthy adults including attention, episodic memory, working memory and executive function, yet previous intervention studies have either only utilised a small number of tasks (Hamazaki et al., 1996), focused on one cognitive domain (Fontani et al., 2005), or using tasks specifically involving emotional information processing (Antypa et al., 2009). In order to address these issues, the present study assessed the effects of two different formulations of fish oil in parallel, and at doses consistent with the current recommended daily intake of oily fish, across a range of cognitive domains. The aim of the present investigation was therefore to specifically evaluate the effects of 12 weeks supplementation of DHA-rich fish oil and EPA-rich fish oil dietary supplements on cognitive function in healthy young adults maintaining a regular diet containing oily fish not more than

once a week. Self-report mood assessments formed the secondary part of this investigation.

4.2 Materials and Methods

4.2.1 Design

A placebo-controlled, double-blind independent measures design was employed with participants randomly assigned to one of three treatment groups (placebo, DHA-rich fish oil, EPA-rich fish oil; see Section 4.2.3). Prior to the start of the study a restricted randomisation (55 X 3 treatments) list matching treatments to participant code numbers was computer generated. Participants were assigned the next available code upon completion of the Training day.

4.2.2 Participants

Volunteers were invited to participate if they declared that they were in good health and that they were a non-smoker, free from prescription, herbal, illicit or recreational drugs (females taking the contraceptive pill were included), omega-3 supplements, that they were a native English speaker and eating no more than one portion of oily fish a week on average (≤ 4 portions/month). All volunteers were either students attending Northumbria University or were university graduates living in Newcastle-upon-Tyne and the surrounding area.

Of the 159 that were enrolled in the study, nine participants did not attend the Baseline session and eight participants were excluded on the basis that their declared average oily fish intake exceeded four portions a month. Two participants withdrew before the last day of the study (Week 12), though in both cases the reasons were unrelated to the intervention (glandular fever, moving home). Two participants had below 50% compliance as they had only taken 1 x 500 mg capsule/d and were excluded from the analysis at the blind data review point. The remaining per-protocol sample consisted of 140 males and females; their demographic details are presented in Table 4.1.

The study was approved by the Northumbria University School of Psychology and Sport Science Ethics Committee and was carried out in accordance with the Declaration of Helsinki (1964). All participants gave written informed consent prior to their inclusion in the study.

Table 4.1. Participant demographic information and baseline characteristics. Means and SEM are given where appropriate. *P* values are given for separate one-way ANOVAs that were conducted on this baseline data.

	Placebo		DHA-rich FO		EPA-rich FO		*p*
N (M/F)	11/37		17/29		18/28		0.193
Age	21.94	*3.66*	21.96	*4.14*	22.74	*3.47*	0.506
BMI	24.83	*3.45*	24.11	*4.12*	23.89	*5.59*	0.572
Average oily fish consumed/month	1.35	*1.37*	1.85	*1.34*	1.20	*1.41*	0.062

4.2.3 Treatments

The 1 g daily dose was provided by 2 x 500 mg capsules. The active capsules contained 497.5 mg of deodorised fish oil (FO) plus 2.5 mg mixed tocopherols. The total daily dose of n-3 PUFAs for the DHA-rich FO was 450 mg DHA + 90 mg EPA (5:1 DHA to EPA) and for the EPA-rich[1] FO this amount was 300 mg EPA + 200 mg DHA (3:2 EPA to DHA). The total daily dose for the placebo treatment was 1 g olive oil. The ratios of DHA and EPA contained in the active treatments are reflective of those that are found naturally; the DHA-rich FO resembles that of yellowfin tuna and herring, and some species of salmon have a similar ratio to that present in the EPA-rich FO. Treatment oils were purchased from EPAX AS (Aalesund, Norway) and encapsulated by Cardinal Health UK. All capsules used a brown bovine gelatine casing and all treatments were packaged, labelled and randomised on site by a disinterested third party.

4.2.4 Assessment of cognitive performance and mood

Performance was assessed using the COMPASS (Computerised Mental Performance Assessment) system, which presented a battery of standard cognitive tasks identical to the battery presented to participants in the previous chapter (see

[1] It is noted that the ratio of EPA:DHA in the EPA 'rich' FO is closer to 1 compared to the DHA-rich FO, however in this case EPA-rich denotes containing more EPA than DHA.

Section 3.2.2.1 for details)**.** All presentations were made via a laptop computer with the exception of the Word Recall and Verbal Fluency tasks, where participants used pen and paper to make their responses. All reaction times were measured in milliseconds. Participants also completed three repetitions of the Cognitive Demand Battery (see Section 3.2.3.2) along with the Bond-Lader visual analogue scales and the DASS (see Section 3.2.4).

4.2.5 Procedure

Participants attended the laboratory on three occasions (Training day, Baseline and Week 12). Testing took place in a suite of testing facilities with participants visually isolated from each other. The Training day followed the exact protocol described in Section 3.2.5 comprising obtaining of informed consent, completing a diet and health questionnaire (Appendix IV), training on the cognitive tasks and mood questionnaires, collection of demographic data and collection of a blood sample. No more than 8 days following the Training day, participants then attended the laboratory at 8:30 a.m. in an overnight fasted state on the first day (Baseline) and at the end of the 12-week treatment regimen (Week 12 – average time on treatment 86.71 days). On arrival to the Baseline session participants were randomised to treatment group and completed the full battery of COMPASS tasks, Bond-Lader visual analogue scales, three repetitions of the CDB and filled in the DASS. Participants' blood pressure and heart rate were subsequently recorded and at the end of the session each participant received their allocated capsules and instructions on how to take them, and asked to commence taking the treatment that day. Participants from whom a blood sample could not be obtained on the Training Day were invited to attempt to give another sample at the end of this session. The session at Week 12 followed exactly the same protocol as at the Baseline session with the exception that all unused capsules were collected prior to testing and at the very end of the session participants filled out a questionnaire regarding their socio-economic status and which treatment they thought they had been taking (Appendix V). The assessment schedule can be found in Figure 4.1.

4.2.5.1 Blood samples

Participants were required to give two blood samples, the first on either the Training Day (*n* = 108) or at the end of the Baseline session (*n* = 3) prior to supplementation and again at Week 12 (*n* = 99) after completing all the behavioural tasks. Blood samples were collected and analysed using the identical methods as described in Chapter 3.2.4.1. In light of the results reported in Chapter 3, in addition to reporting serum concentrations of DHA and EPA in the present study, the ratio of AA:EPA is also presented in Table 4.2.

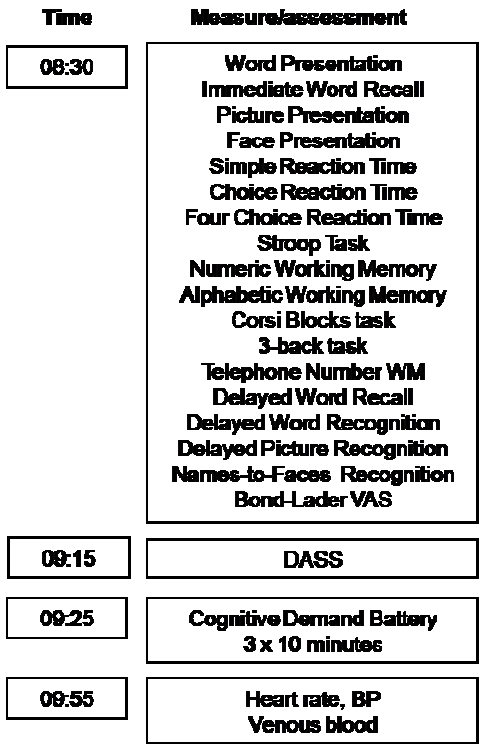

Figure 4.1. Baseline and Week 12 assessment schedule. The pre-treatment venous blood sample was collected on either the Training day or at Baseline. Times are approximate.

4.2.5.2 Statistical methods

Data were analysed by between subjects (treatment group) Analysis of Covariance (ANCOVA) with data from the relevant outcome measure at Baseline as the covariate. *A priori* planned comparisons were made between placebo and both the active treatments (DHA-rich FO, EPA-rich FO) utilising t tests with the mean

squares error from the ANCOVA as an error term and adjusted means (Keppel, 1991). To ensure the overall protection level against Type I errors, comparisons were strictly planned prior to the commencement of the study and only those comparisons associated with a significant main effect on the omnibus ANCOVA were calculated. All testing was two-tailed and all data were analysed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

4.2.5.2.1 Baseline characteristics

Results from separate between groups (treatment condition) ANOVAs using data collected on either the Training day or Baseline session revealed no differences between participants in all three treatment groups on age, BMI, oily fish consumption (see Table 4.1), and serum concentrations of DHA, EPA (see Table 4.2). Additionally, no between group differences were detected on breastfeeding, white fish consumption, and any of the family background questionnaire items excepting mother's level of education (M-Ed). For this item the ANOVA revealed a significant main effect of treatment [$F(2, 137) = 3.91$, $p = 0.022$]. Consequently all of the separate one-way ANCOVAs of the data collected at Week 12 were carried out twice, once using the appropriate outcome from the Baseline session and M-Ed as covariates, and once without. Given that M-Ed inconsistently predicted the outcome variable and the results from both sets of analyses were identical as regards main effects, M-Ed was not included as a covariate in the main analysis. There was also a trend towards a difference between groups on average oily fish consumed ($p = 0.062$), with participants in the DHA-rich FO treatment group reporting higher average consumption.

4.2.5.2.2 Missing data

Data capture errors lead to a reduced number of datasets for the Corsi Blocks task and two participants' data were lost from the entire CDB due to a fire drill during their Baseline testing session. During the data cleaning process (conducted prior to treatment unblinding), it was evident that some participants had either not understood or followed the directions properly (e.g. performance on a task that was at chance or consistent with use of a single button, coupled with large variations in

reaction times suggesting inattention to the stimuli). Therefore reduced datasets were also used in the analysis of the Stroop, Serial 7 subtractions and RVIP tasks, and are reflected in Tables 4.3 and 4.4. For the subsequent ANCOVAs that were carried out on the outcomes from these tasks, missing data were omitted using listwise deletion.

4.3 Results

4.3.1 Physiological data and compliance

Results for compliance as measured by capsule count were similar in all 3 treatment conditions (91% DHA-rich FO, 90% EPA-rich FO, and 92% Placebo). Reference to the serum fatty acid data presented in Table 4.2 shows that serum concentrations of DHA and EPA increased significantly in both treatment groups but not in the placebo group. More specifically, fatty acid concentrations of DHA increased by 48% and EPA increased by 30% in the DHA-rich FO group and for those taking the EPA-rich FO, concentrations of DHA and EPA increased by 33% and 53%, respectively. Paired samples t-tests were conducted to analyse the change in serum DHA and EPA in all three treatment groups, results of which are presented in Table 4.2.

Table 4.2. Physiological measures by treatment group at Baseline and Week 12. Means and SEM are presented. For serum concentrations of DHA and EPA (%total FAME) and the ratio between concentrations of AA and EPA significant difference between Baseline and Week 12 concentrations (paired samples t-test) is indicated in bold typeface (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$).

		Baseline			Week 12		
		n	Mean	SEM	n	Mean	SEM
DHA	Placebo	37	1.05	0.07	32	0.95	0.08
	DHA-rich FO	37	1.26	0.09	34	**1.87*** **	0.11
	EPA-rich FO	38	1.12	0.09	33	**1.49***	0.11
EPA	Placebo	37	1.07	0.09	32	1.31	0.12
	DHA-rich FO	37	1.05	0.06	34	**1.36****	0.12
	EPA-rich FO	38	1.16	0.09	33	**1.78****	0.12
AA:EPA	Placebo	37	8.03	0.45	32	**10.66*** **	0.73
	DHA-rich FO	37	7.74	0.39	34	**4.46****	0.31
	EPA-rich FO	38	6.96	0.42	33	7.74	0.43
Systolic BP	Placebo	48	62.98	1.62	48	64.88	1.37
	DHA-rich FO	46	63.28	1.84	46	62.37	1.57
	EPA-rich FO	46	61.37	1.46	46	63.50	1.43
Diastolic BP	Placebo	48	113.00	1.61	48	114.71	1.86
	DHA-rich FO	46	119.28	1.87	46	120.72	1.89
	EPA-rich FO	46	117.30	1.64	46	116.80	1.91
Heart rate	Placebo	48	77.08	2.28	48	75.04	1.14
	DHA-rich FO	46	79.15	1.64	46	76.74	1.45
	EPA-rich FO	46	77.28	2.00	46	77.96	1.24

In a separate set of analyses, data from the serum samples collected at Week 12 were analysed by one-way ANCOVA with relevant serum data collected at Baseline as the covariate to investigate the presence of between group differences on serum PUFAs following the intervention. A significant main effect of treatment group was revealed for serum concentrations of both DHA and EPA [$F(2, 85) = 18.10$, $p < 0.001$; $F(2, 84) = 3.24$, $p = 0.044$, respectively]. *Post hoc* calculations (Bonferroni) revealed that participants in the DHA-rich FO group had higher serum DHA than placebo ($p < 0.001$) and those in the EPA-rich FO group ($p = 0.022$). Those in the EPA-rich FO group also had higher serum DHA than placebo ($p = 0.004$). Interestingly, as regards serum EPA concentrations, *post hoc* calculations did not reveal any significant differences between the treatment groups and placebo, though there was a trend for higher serum EPA in the EPA-rich FO group compared to the DHA-rich FO group ($p = 0.080$) and placebo ($p = 0.097$).
The same analysis was applied to the AA:EPA ratio data and a significant main effect of group was also detected [$F(2, 84) = 33.61$, $p < 0.001$]. The ratio of AA:EPA was higher in the placebo group than both the DHA-rich FO ($p < 0.001$) and EPA-rich FO ($p < 0.001$) groups. The was no significant difference between the active treatment groups on the ratio of AA:EPA.

At the end of the Week 12 session participants were asked to say if they thought they had been taking fish oil or placebo capsules; participants were just as likely to give the correct answer in the EPA-rich FO treatment group (49%) as those taking placebo (66%) [χ^2 (1) = 0.08; $p = 0.109$] whereas those in the DHA-rich FO group were less likely (37%) to give the correct answer [χ^2 (1) = 0.003; $p = 0.006$].

As regards the other physiological measures that were taken, there was no evidence of an effect of treatment on either blood pressure or heart rate. These data can also be found in Table 4.2.

4.3.2 Cognitive performance battery (COMPASS)

Performance data collected at Baseline and Week 12 data for every outcome can be found in Table 4.3.

There was evidence of a significant main effect of treatment on two of the outcome measures including reaction time on the Stroop task [$F(2, 133) = 3.27$, $p = 0.041$]

and number of correctly matched items on the Names-to-Faces task [$F(2, 136) = 3.73$, $p = 0.026$]. Planned comparisons revealed that participants in the DHA-rich FO group were faster on the Stroop task compared to placebo [$t(133) = 2.50$, $p = 0.014$, $r = 0.26$] but participants in both the DHA-rich and EPA-rich FO groups matched fewer items on the Names-to-Faces task [$t(136) = 2.00$, $p = 0.047$, $r = 0.21$; $t(136) = 2.52$, $p = 0.013$, $r = 0.26$, respectively].

Table 4.3. Cognitive task performance and mood outcomes by treatment group at Baseline and Week 12. Means and SEM are presented with *F* and *p* values from the primary ANCOVA. Significant results are presented in bold typeface.

		Baseline			Week 12				
		n	Mean	SEM	*n*	Mean	SEM	*F*	*p*
Immediate Word Recall (no. correct)	Placebo	48	6.99	0.36	48	7.79	0.36		
	DHA-rich FO	46	6.90	0.30	46	7.17	0.30	1.39	0.252
	EPA-rich FO	46	7.33	0.33	46	7.59	0.31		
Delayed Word Recall (no. correct)	Placebo		5.08	0.41		5.93	0.32		
	DHA-rich FO		4.71	0.37		5.61	0.35	0.05	0.949
	EPA-rich FO		5.00	0.33		5.87	0.31		
Simple RT	Placebo		281.16	3.76		285.07	3.71		
	DHA-rich FO		289.56	5.20		288.52	5.14	0.13	0.880
	EPA-rich FO		282.37	4.35		283.44	4.16		
Choice RT	Placebo		399.49	6.05		410.65	9.90		
	DHA-rich FO		403.15	8.51		399.59	8.21	1.02	0.365
	EPA-rich FO		396.88	6.28		404.37	11.30		
Choice RT (% accuracy)	Placebo		94.88	0.53		95.71	0.47		
	DHA-rich FO		95.30	0.56		95.78	0.44	1.45	0.238
	EPA-rich FO		94.65	0.65		94.70	0.55		
4 Choice RT	Placebo		464.35	6.67		464.78	6.35		
	DHA-rich FO		482.41	10.66		470.22	9.72	1.20	0.310
	EPA-rich FO		479.96	8.73		481.22	11.64		
4 Choice RT (% accuracy)	Placebo		99.05	0.21		99.18	0.18		
	DHA-rich FO		98.55	0.26		98.96	0.28	0.64	0.527
	EPA-rich FO		98.69	0.34		98.69	0.32		
Stroop (RT)	Placebo		817.44	13.33	47	855.68	16.44		
	DHA-rich FO	45	858.44	20.43	44	845.02	17.23	**3.27**	**0.041**
	EPA-rich FO		843.00	17.21		863.87	20.64		
Stroop (% accuracy)	Placebo		99.38	0.16		99.40	0.16		
	DHA-rich FO		99.33	0.17		99.14	0.26	0.43	0.655
	EPA-rich FO		99.00	0.27		99.26	0.18		
Verbal Fluency	Placebo		38.64	0.38		41.66	1.24		
	DHA-rich FO		39.70	0.44		41.21	1.46	1.46	0.236
	EPA-rich FO		41.17	0.42		41.97	1.09		
Numeric Working Memory (RT)	Placebo		809.48	22.20		844.76	24.11		
	DHA-rich FO		829.36	24.40		877.76	26.92	0.61	0.545
	EPA-rich FO		797.41	22.44		828.38	20.38		
Numeric Working Memory (% accuracy)	Placebo		96.09	0.64		96.85	0.42		
	DHA-rich FO		96.40	0.41		95.99	0.54	1.31	0.270
	EPA-rich FO		95.53	0.61		95.36	0.87		
Alphabetic Working Memory (RT)	Placebo		466.10	12.66		479.76	12.86		
	DHA-rich FO	39	512.37	16.22		511.30	14.84	0.02	0.978
	EPA-rich FO		459.46	13.59		472.33	13.65		
Alphabetic Working Memory (% accuracy)	Placebo		94.65	0.52		94.40	0.54		
	DHA-rich FO		94.28	0.56		94.52	0.69	0.05	0.949
	EPA-rich FO		94.52	0.71		94.57	0.58		
Corsi Blocks Span	Placebo		6.01	0.16		5.99	0.17		
	DHA-rich FO		6.18	0.15		6.07	0.23	0.20	0.818
	EPA-rich FO		5.64	0.21		5.81	0.25		
3-back task (RT)	Placebo	45	1053.69	71.79		1020.13	78.79		
	DHA-rich FO		1146.98	98.39		1224.29	110.00	2.65	0.075
	EPA-rich FO	37	1037.16	99.48		1196.88	111.93		

		Baseline			Week 12				
		n	Mean	SEM	*n*	Mean	SEM	*F*	*p*
3-back task (% accuracy)	Placebo		84.68	1.95		80.60	2.76		
	DHA-rich FO		81.52	2.63		82.83	2.36	1.96	0.145
	EPA-rich FO	45	80.21	2.52		81.43	2.44		
Telephone Number task (RT)	Placebo		5997.14	237.84		5758.90	204.10		
	DHA-rich FO		6578.48	259.07		6739.05	276.45	2.39	0.095
	EPA-rich FO		6624.08	280.40		6588.26	297.23		
Telephone Number task (% accuracy)	Placebo		35.42	3.87		35.16	3.99		
	DHA-rich FO		38.86	3.83		36.14	3.50	0.09	0.915
	EPA-rich FO		38.59	3.80		37.77	3.82		
Word Recognition (RT)	Placebo		924.44	28.81		872.85	24.37		
	DHA-rich FO		892.43	21.04		894.11	24.64	2.72	0.070
	EPA-rich FO		897.83	31.26		916.02	25.51		
Word Recognition (% accuracy)	Placebo		77.36	1.67		81.81	1.42		
	DHA-rich FO		75.36	1.46		79.35	1.53	0.42	0.659
	EPA-rich FO		78.77	1.44		81.96	1.30		
Picture Recognition (RT)	Placebo		954.67	34.89		992.96	32.70		
	DHA-rich FO		972.35	57.54		1053.98	38.59	1.70	0.187
	EPA-rich FO		933.63	29.59		1067.30	32.80		
Picture Recognition (% accuracy)	Placebo		88.40	1.47		83.40	1.65		
	DHA-rich FO		89.42	1.29		86.16	1.58	0.64	0.529
	EPA-rich FO		91.96	0.79		87.17	1.40		
Names-to-Faces Recall (no. correct)	Placebo		11.21	0.69		13.90	0.73		
	DHA-rich FO		11.98	0.62		12.67	0.60	**3.73**	**0.026**
	EPA-rich FO		12.07	0.55		12.26	0.68		
Bond-Lader Alert	Placebo		49.78	2.05		53.62	2.15		
	DHA-rich FO		50.59	1.76		52.47	2.12	0.14	0.871
	EPA-rich FO		48.28	1.82		52.46	2.21		
Bond-Lader Calm	Placebo		59.68	1.62		62.58	1.82		
	DHA-rich FO		58.05	1.88		60.64	1.77	0.12	0.901
	EPA-rich FO		57.02	1.76		60.23	2.09		
Bond-Lader Content	Placebo		59.40	1.68		61.07	1.74		
	DHA-rich FO		57.87	1.86		61.98	1.74	0.39	0.680
	EPA-rich FO		58.73	1.58		60.65	1.97		
DASS Stress	Placebo		9.27	0.98		7.92	0.96		
	DHA-rich FO		7.74	0.85		7.98	0.93	0.52	0.595
	EPA-rich FO		8.07	1.01		7.24	0.93		
DASS Anxiety	Placebo		3.42	0.62		3.38	0.64		
	DHA-rich FO		3.54	0.56		3.24	0.50	0.21	0.808
	EPA-rich FO		2.65	0.56		3.09	0.60		
DASS Depression	Placebo		4.63	0.76		3.27	0.82		
	DHA-rich FO		5.54	0.94		4.52	0.82	0.40	0.672
	EPA-rich FO		4.41	0.79		3.67	0.65		

4.3.3 Cognitive Demand Battery (CDB)

Performance data collected at Baseline and Week 12 on the CDB can be found in Table 4.4.

There was no evidence of a main effect of treatment on any of the cognitive performance measures from the CBD for either ANCOVA model. However a main effect of treatment was detected using both models for self-reported mental fatigue during the CDB [F (2, 133) = 3.27, p = 0.041]. Planned comparisons revealed that mental fatigue scores for participants in the EPA-rich FO group were significantly lower compared to placebo [t(134) = -2.67, p = 0.009, r = 0.27].

Table 4.4. Outcome measures on the Cognitive Demand Battery by treatment group at Baseline and Week 12. Means and SEM are presented with F and p values from the primary ANCOVA. Significant results are presented in bold typeface.

		Baseline			Week 12				
		n	Mean	SEM	*n*	Mean	SEM	*F*	*p*
Serial 3 Subtractions (no. correct)	Placebo	47	38.05	2.09	47	37.55	2.01	0.08	0.925
	DHA-rich FO	45	35.86	2.02	45	35.51	1.97		
	EPA-rich FO	46	36.12	1.90	46	35.53	1.76		
Serial 7 Subtractions (no. correct)	Placebo	48	21.49	1.51	46	21.40	1.48	0.15	0.863
	DHA-rich FO	44	21.11	1.46	45	20.99	1.55		
	EPA-rich FO	46	20.61	1.39	46	20.99	1.31		
RVIP (RT)	Placebo	47	484.87	9.20	44	485.64	10.23	0.12	0.897
	DHA-rich FO	44	492.03	8.51	41	481.37	8.99		
	EPA-rich FO	42	473.61	7.14	43	475.46	9.53		
RVIP (% accuracy)	Placebo	47	50.83	3.30	44	50.31	3.69	1.48	0.232
	DHA-rich FO	44	49.50	3.45	41	51.68	3.34		
	EPA-rich FO	42	53.40	3.26	43	55.33	3.73		
RVIP (false alarms)	Placebo	47	8.14	1.63	44	8.55	1.59	0.35	0.705
	DHA-rich FO	44	6.58	1.04	41	6.76	1.04		
	EPA-rich FO	42	8.50	1.63	43	10.36	2.38		
Mental fatigue (/100)	Placebo	48	66.75	2.32	47	68.78	2.02	**4.36**	**0.015**
	DHA-rich FO	46	61.67	2.16	46	65.35	2.54		
	EPA-rich FO	46	63.93	2.29	45	59.36	2.67		

4.3.4 Mood

There was no effect of treatment on either the Bond-Lader visual analogue scales or on the DASS. The scores from both these measures can be found at the bottom of Table 4.3.

4.4 Discussion

Of the cognitive performance tasks, it was found that a faster reaction time was achieved by participants in the DHA-rich FO group on the Stroop task. It was also found that participants receiving both the active treatments matched fewer names to faces in the Name-to-Faces Recognition task. In addition, there was no effect of either treatment on any of the Bond-Lader or DASS mood measures, but participants administered the EPA-rich FO reported reduced mental fatigue compared to placebo during thirty minutes of cognitively demanding tasks. Given the number of different tasks, along with the small number of significant outcomes that were detected and the bi-directional nature of the results, overall these findings do not provide solid evidence of cognitive or mood enhancement following supplementation with either active treatment for 12 weeks.

The serum blood analysis revealed that both doses of DHA contained in the DHA-rich FO (450 mg) and the EPA-rich FO (200 mg) was sufficient to raise serum DHA above that of participants in the placebo group at Week 12. However neither dose of EPA (300 mg - EPA-rich FO; 90 mg – DHA-rich FO) achieved this despite the active treatments being successful in increasing within-group serum concentrations of EPA at Week 12 compared to Baseline concentrations. As regards the other physiological measures, there was no evidence of an effect of either of the active treatments on heart rate or blood pressure. This is in agreement with a meta-analysis reported by Morris et al. (1993) who found no effect of n-3 PUFA supplements on blood pressure in healthy adults. More recently Shah et al. (2007) reported that 1 g/d fish oil for 14 days reduced resting heart rate and improve endothelial function in healthy men and women, though the sample was much smaller (N = 26) with a higher mean age (31 years) than the current study, which could potentially account for the differences.

Whilst the current results provide little evidence of cognitive enhancement following n-3 PUFA supplementation, there are some interesting parallels with findings from studies in similar samples. For example, the reduced reaction time on the Stroop task observed in the DHA-rich FO group is consistent with Fontani et al. (2005) who reported a beneficial within-treatment effect of fish oil on a go/no-go task and Rogers et al. (2008) who reported a trend indicating improved performance on a task of impulsivity following twelve weeks supplementation with a DHA-rich FO in mild to moderately depressed adults. One hypothesis that could be drawn from the

available evidence is that tasks requiring response inhibition may be sensitive to increased intake of n-3 PUFAs. In addition, taking into account the results of the present study it could be added that increased intake of DHA and not EPA may responsible for the observed improvement on the Stroop task, considering the same finding was not observed in the EPA-rich FO group, where the amount of DHA contained in the supplement was less than half of that contained in the DHA-rich FO (200 mg as opposed to 450 mg). Although mechanisms that might mediate cognitive performance cannot be discerned from the present results, one potential avenue to explore in future investigations is modulation of dopaminergic neurotransmission by DHA-rich FO. Dopamine is known to modulate response inhibition (Le Moal and Simon, 1991), and research in both animals and humans has linked changes in n-3 PUFA intake to alterations in the dopaminergic system. For example, n-3 PUFA deficiency in rats has previously been associated with reduced levels of dopamine and dopamine D2 receptors in the frontal cortex (Delion et al., 1996), to which behavioural changes including increased activity and reduced motivation observed in n-3 deficient animals have been attributed (Zimmer et al., 2002). Further, Chalon et al. (1998) revealed that n-3 PUFA supplementation increased endogenous cortical dopamine levels in rats and Hershey et al. (2004) demonstrated the involvement of dopaminergic neurotransmission in response inhibition in healthy adults; administration of Levodopa—a dopamine agonist—modulated activation of go/no-go task-specific areas of the brain. Altogether this evidence may suggest a role for n-3 PUFAs, and DHA in particular, in tasks requiring response inhibition, though this would need to be investigated further. One last point to note is that in contrast to the finding reported here, Hamazaki et al. (1996) did not report an effect of a high DHA oil supplement on a Stroop task. The version of the task used in their study was modified, however, so that participants were required to respond using a pen and paper. One possibility is that responses were less accurately recorded compared to the computerised version utilised here.

Another parallel with previous research concerns the beneficial effect of the EPA-rich FO on self-reported mental fatigue. This finding is consistent with previous reports revealing reduced ratings of mental fatigue on the Profile of Mood States following FO supplementation for 4 weeks in healthy adults (Antypa et al., 2009, Fontani et al., 2005). What is interesting is that the average serum concentration of EPA collected at Week 12 in the EPA-rich FO treatment group was not significantly different from placebo. However, reference to the serum AA:EPA data shows that

this ratio was significantly higher in the placebo group and had in fact increased in Week 12 compared to Baseline. It is therefore possible to speculate that the underlying mechanism responsible for the reduced mental fatigue reported by those in the EPA-rich FO group might be modulation of the production of either AA or EPA-derived eicosanoids, known to be regulators of many key immune, endocrine and cardiovascular functions (Tassoni et al., 2007). This hypothesis is also consistent with the results reported in Chapter 3, where the ratio of AA:EPA was positively associated with mental fatigue on the same battery of cognitively demanding tasks. Together these results suggest that mental fatigue could be linked to an endogenous balance of AA and EPA, and that reducing this ratio or preventing it from elevating via increased intake of n-3 PUFAs may have a beneficial impact on subjective reporting of mental fatigue. An interesting point to note is that despite reduced reporting of mental fatigue, there was no observable benefit of either intervention on the performance outcome measures of CDB. This could be an artefact of the task difficultly, with participants disengaging with the tasks, or simply that neither treatment modulates performance on these tasks. A further note is that the same effect of reduced mental fatigue was not observed in the DHA-rich FO condition where the serum AA:EPA ratio of participants in this group was also significantly lower than that of the placebo group. However, the daily dose of EPA in the DHA-rich FO was less than a third of that present in the EPA-rich FO (90 mg as opposed to 300 mg), so it is possible that this may be a contributing factor, even though serum concentrations of EPA were not significantly different from placebo at Week 12 in the EPA-rich FO group. On this point it must be noted that the failure of the EPA-rich FO to raise participants' serum concentrations of EPA above that of the placebo group is a limitation of this study that would need to be addressed in future studies. Given that serum concentrations of EPA were found to be significantly positively associated with performance on a cluster of episodic memory tasks in Chapter 3 suggests that the possibility of enhancement on these tasks being observed following higher doses, although unlikely, cannot be ruled out.

Returning briefly to the mental fatigue data, a related observation concerns the fact that participants who were administered the active treatments were not able to correctly identify if they had been taking fish oil or placebo capsules. Of those that did guess correctly, none listed feeling less fatigued as a reason, regardless of group, so the reduced mental fatigue experienced by participants taking the EPA-

rich FO induced under laboratory conditions may have been too subtle to have an impact on everyday functioning. One future extension could evaluate the efficacy of EPA-rich FO to ameliorate symptoms of fatigue, both mental and physical, in populations who subjectively report fatigue such as individuals in full-time work, primary caretakers of children, and carers, for example.

Also in relation to the serum AA:EPA ratio data reported here, it is potentially surprising that no effect of treatment was detected on any of the subscales of the DASS, especially given the significant positive association between anxiety and serum AA:EPA reported in Chapter 3. Previously, n-3 PUFA supplements have been shown to be effective at reducing feelings of anxiety in substance abusers (Buydens-Branchey and Branchey, 2006), and DHA-rich fish oil reduced levels of noradrenalin—a hormone mediator of the stress response—in students under continued exam stress (Hamazaki et al., 1999). Considering that psychological stress and anxiety are also linked to elevated pro-inflammatory cytokine profiles (Maes et al., 1998), which are in turn modulated by dietary intake of n-3 PUFAs (Ferrucci et al., 2006), future research could incorporate paradigms that induce psychological stress or anxiety akin to the laboratory-induced mental fatigue achieved by the CDB, as a more appropriate evaluation of the efficacy and underlying mechanisms of n-3 PUFAs on these outcomes. As it stands these data presented here do not suggest that DHA-rich or EPA-rich FO modulates stress, anxiety or depression as measured by the DASS in healthy young adults.

Fish oil supplementation was also associated with some cognitive costs. Both treatment conditions were associated with poorer performance on the Names-to-Faces Recall task. To the author's knowledge this is the first reporting of a detrimental effect of fish oil or n-3 PUFA supplementation on cognitive function and there is no theoretical basis that would support these particular observations. In addition, Chapter 3 reported a positive association between serum concentrations of EPA and total n-3 PUFAs and number of correctly matched items on this task (see Table 3.2), which is not consistent with this finding. As previously mentioned however, a limitation of this study is the large number of tasks that were utilised, which generated an even larger number of separate outcomes. It is possible therefore that this negative finding is a result of Type I error. As such, this and all the results reported in the current chapter should be interpreted with caution, however the decision to use many different performance measures can be

defended considering the findings of the study described in Chapter 3, where serum fatty acid levels were associated with performance across a number of different cognitive domains, rendering it difficult to specifically select tasks where an effect of treatment could be predicted.

On a similar note it should be emphasised that significant benefits of supplementation were only observed on a single outcome on the COMPASS battery. Animal studies that have administered supplemental n-3 PUFAs to rats raised on a balanced and n-3 adequate diet performed no better on behavioural tests than the animals given no supplement (de Wilde et al., 2002), suggesting that performance cannot be enhanced when n-3 PUFAs are supplemented to excess. In the present study, a sample of healthy adults was recruited, selected only on the basis of not consuming a high amount of oily fish (≤ one portion of oily fish a week on average), with a view to providing information about the effects of supplementation in an otherwise unselected population about which very few data existed. As such, the results presented here suggest that performance benefits incurred from dietary supplementation with fish oils in a healthy unselected population at a dose that is roughly equivalent to the current UK recommendation (SACN/COT, 2004), are minimal. One possible interpretation of the findings could be that even though three quarters of the present sample reported consuming less oily fish than the current recommendation, and despite the fact that serum concentrations of DHA at least were generally lower than what has been observed in normal healthy participants in previous studies (Morse, 2009), endogenous n-3 PUFA levels were not below a certain threshold where performance deficits are observed and can be subsequently improved when n-3 PUFAs are administered. One particular study in animals does support the suggestion of a threshold of n-3 PUFA intake (and subsequent cellular incorporation), below which performance is adversely affected. Jensen et al. (1996) compared the effects of four different diets on brain phospholipids and performance in rats on the Morris Water Maze (MWM) task after four generations of dietary intervention. The diets were seal oil (rich in DHA and EPA, but containing less EPA than fish oil), fish oil (rich in DHA and EPA), flaxseed oil (containing the parent n-3 fatty acid α-linolenic acid; ALA) and normal rat chow containing 2% fat with a mixture of ALA and linoleic acid (n-6). These authors report that when brain levels of n-3 PUFAs (DHA + docosapentaenoic acid) contained in phosphatidylethanolamine were between 24 and 27% as they were in the rats fed vegetable and marine oils, there was no difference in performance on

the MWM, however when these levels fell to 22%, as they were in the control rats fed the normal chow, performance was worse, with significantly longer escape latencies. As regards the current study, more significant and notable improvements in performance may be seen, perhaps, if a sample were selected on the basis of non-consumption of oily fish, and also at higher dosages, considering ineffectual modulation of serum EPA levels by both treatments. It is also possible that benefits of n-3 PUFA supplementation on cognitive function, if they indeed exist in healthy populations, may be better observed at times when resources are in high demand, considering the beneficial effect of the EPA-rich FO on mental fatigue reported here. One potential future paradigm could possibly reverse the order of the test batteries, so that mental fatigue was induced prior to further cognitive testing with the COMPASS battery.

The current study describes the first investigation where the effects of DHA- and EPA-rich fish oils on cognitive function and mood have been investigated in parallel under the exact same conditions. The primary aim of this study was to assess the effects of n-3 PUFA supplementation in a population where few data are available regarding cognitive performance and mood. A 1 g dose of FO was chosen so that the results could be readily translated into meaningful dietary advice. As such, the data presented here do not favour the use of fish oil supplements for cognitive or mood enhancement.

CHAPTER 5. CEREBRAL HEMODYNAMIC EFFECTS OF DHA-RICH FISH OIL AND EPA-RICH FISH OIL IN HEALTHY YOUNG ADULTS

5.1 Introduction

The n-3 PUFAs DHA and EPA, abundantly found in oily fish are essential and must be supplied via dietary sources. In the brain, DHA is required to maintain the structural integrity of neuronal cell membranes, and comprises approximately 10-20% of fatty acids found in this organ (McNamara and Carlson, 2006). The presence of DHA in cell membranes increases membrane fluidity and permeability, which in turn can impact upon signal transduction pathways (see Section 1.4.1). EPA, though not a major component of the brain, could potentially influence brain physiological function and therefore behaviour via the action of its many eicosanoid derivatives; regulators of key immune, endocrine and cardiovascular functions (see Section 1.4.2). Animal studies have demonstrated that when n-3 PUFAs are absent from the diet, cognitive deficits are observed, compared to n-3 adequate control animals (see Section 1.5.1). In humans, low n-3 PUFA status is associated with a number of adverse health and behavioural outcomes, however n-3 PUFA intervention trials have provided mixed results with some studies showing a benefit of n-3 PUFAs on symptom reduction or behavioural measures, while others do not (see Sections 1.5.2.1 and 1.5.2.3). More recently, the issue of cognitive and mood enhancement via n-3 PUFA supplementation has been explored in healthy volunteers (e.g. Dalton et al., 2009, Fontani et al., 2005, Hamazaki et al., 1996, Hamazaki et al., 1998, Itomura et al., 2005, Osendarp et al., 2007, Rogers et al., 2008, Chapter 2, Chapter 4), but again these studies have not yielded conclusive findings overall. Despite the relative lack of consistent data regarding the behavioural effects of dietary n-3 PUFAs, it is possible that n-3 PUFAs are still impacting upon various physiological functions of the brain. For example, the DHA content of the brain is indeed influenced by dietary intake; in animals DHA supplementation has been shown to increase levels of this fatty acid in the brain (Moriguchi et al., 2001, Connor et al., 1990). In contrast, reduced dietary DHA in rats is reflected in lower membrane phospholipid levels in the brain (fronto-parietal cortex, hippocampus and suprachiasmatic nucleus), which appears to be related to

reductions in regional metabolic rate (da Silva et al., 2002). Further, one study in aged rhesus monkeys (mean age 18 years) discovered that DHA supplementation for four weeks restored age-related cerebral blood flow response to tactile stimulation (Tsukada et al., 2000a).

Similarly, in humans n-3 PUFA intake (from two 24-hr food recall interviews) has been shown to be positively associated with grey matter volume in corticolimbic areas (Conklin et al., 2007), however to the author's knowledge only three studies have directly examined the relationship between n-3 PUFAs and physiological brain function. The first discovered that administration of n-3 PUFAs (either 8.4 g or 2 g DHA+EPA) for 4 weeks in patients with bipolar disorder resulted in decreased whole-brain T2 relaxation times (as measured by fMRI[2]), compared to placebo, which the authors interpreted as reflecting increased membrane fluidity due to incorporation of n-3 PUFAs in neuronal cell membranes (Hirashima et al., 2004). Another study reported that fish oil supplementation (1.60 g EPA + 0.80 g DHA) resulted in increased amplitude of the negative peak preceding and the positive peak following stimuli on a go/no-go task (EEG; CNV and P300, respectively), though only within-group comparisons were made before and after treatment (Fontani et al., 2005). More recent work has revealed that plasma levels of PUFAs (DHA and AA) were correlated with metabolic activity in a number of brain regions in depressed patients using PET (Sublette et al., 2009). More specifically, DHA and AA were positively associated with glucose metabolism (rCMRglu) in the temporoparietal region and DHA was negatively correlated with rCMRglu in the frontal cortex and anterior cingulate cortex. Taken together, evidence from both animal and human studies suggests that brain physiology and function are associated with dietary n-3 PUFAs, although the nature of this relationship is only beginning to be investigated.

One viable approach to evaluate the effect of dietary n-3 PUFAs on brain physiological function is by measuring cerebral hemodynamics using functional Near Infrared Spectroscopy (NIRS), a non-invasive optical imaging technique. The guiding principle of NIRS is that the chromophores oxyhaemoglobin (O2Hb) and deoxyhaemoglobin (HHb) absorb light at different wavelengths. Therefore, by

[2] Increased T2 relaxation time is indicative of decreased activity in a brain region (Anderson et al., 2002)

measuring the amount of light absorbance at a specific wavelength, the concentrations of O2Hb and HHb can be calculated (Fallgatter and Strik, 1997). Total haemoglobin (THb) is the third outcome to be produced by NIRS measurements and is simply the sum of O2Hb and HHb. In this way NIRS can been used as a measure of activation of neural tissue or simply as a measure of cerebral blood flow. THb is closely related to cerebral blood volume (CBV), from which changes in regional CBF (rCBF) can be inferred (Steinbrink et al., 2005). THb also correlates well with rCBF changes as measured using the labelled-water technique (Villringer and Dirnagl, 1995), although studies using rat brain models suggest that O2Hb is a better measure of rCBF than THb (Hoshi et al., 2001). Changes in concentrations of either O2Hb and HHb are related to changes in cerebral metabolic rates (Tamura et al., 1997), and the concentration of HHb and to a lesser extent O2Hb, is strongly correlated with the fMRI BOLD signal (Huppert et al., 2006). NIRS has been successfully used to image activation in the frontal cortex (e.g. Fallgatter and Strik, 1997, Fallgatter and Strik, 1998) and other areas of the brain including the temporal, visual and parietal cortices (e.g. Schecklmann et al., 2008, Jasdzewski et al., 2003). The use of NIRS in pharmacological interventions is a novel application of this technique, with only a small number of trials published to date (e.g. Kanamaru et al., 2008, Bonoczk et al., 2002). Using NIRS in this way, the opposite effects for a vasodilator (resveratrol - Kennedy et al., 2010) and a vasoconstrictor (caffeine - Haskell and Kennedy, In preparation) have been demonstrated. In comparison with fMRI, the current 'gold standard' for measuring brain activation, NIRS has the advantage of being safe, non-invasive and inexpensive (Bunce et al., 2006). It is also highly portable, and allows for neuroimaging in a more natural setting (Hoshi, 2007). On the other hand, a disadvantage of the NIRS imaging technique is that it has low spatial resolution in comparison to other methods, however it does have the benefit of providing high temporal resolution (Obrig and Villringer, 2003). Finally, because the apparatus is easily and quickly applied to the head, this a valuable tool in studies that require a large number of participants or testing sessions.

To date only a limited number of studies have assessed the relationship between n-3 PUFAs and physiological brain function in humans. Given that dietary supplementation with n-3 PUFAs has previously been shown to modulate fatty acid concentrations in the frontal cortex (e.g. Connor et al., 1990), this area was selected as the region of interest for the current study. In addition, although the extant

literature has primarily focused on the mechanisms of action of DHA in relation to brain function given the high proportion of this fatty acid to be found there, it is also possible that EPA could affect cerebrovascular parameters, given that this fatty acid is also readily incorporated into endothelial cell membranes elsewhere in the cardiovascular system, albeit to a lesser degree than DHA (Hashimoto et al., 1999). Therefore, the objective of this pilot trial was to investigate any treatment related effects upon task-related cerebral hemodynamic response, in the prefrontal cortex following a DHA-rich and an EPA-rich fish oil supplement in healthy volunteers.

5.2 Materials and Methods

5.2.1 Design

A placebo-controlled, double-blind independent measures design was employed with participants randomly assigned to one of three treatment groups (placebo, DHA-rich fish oil, EPA-rich fish oil; see Section 4.2.3).

5.2.2 Participants

Twenty-two healthy adults took part in the study; demographic details can be found in Table 5.1. All participants declared they were in good health, a non-smoker, free from prescription medication and social drugs, free from omega-3 supplements and a native English speaker. Participants also declared they did not consume more than 1 portion of oily fish per week. At the time of testing, participants were enrolled in a larger intervention trial (see Chapter 4) and were invited to take part in an extra testing session once they had completed their Week 12 cognitive assessment.

The study received approval from the Northumbria University School of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent prior to their inclusion in the study.

Table 5.1. Participant demographic data by treatment group. Means and SEM are presented.

	Placebo		DHA-rich FO		EPA-rich FO	
N (Male/Female)	2/5		4/3		3/5	
Age	22.43	1.54	20.71	0.78	22.75	1.83
BMI	23.29	1.12	22.94	0.86	22.88	1.89

5.2.3 Treatments

The three treatments (DHA-rich FO, EPA-rich FO, placebo) used in the current study were identical to those described in Section 4.2.3. Participants were instructed to consume 2 x 500 mg capsules per day for 12 weeks.

5.2.4 Physiological Measures

5.2.4.1 Near Infrared Spectroscopy (NIRS)

Relative changes in the absorption of near infrared light were measured using a 2-channel continuous wave Oxymon system (Artinis Medical Systems B.V.). This type of system applies continuous light to tissue and allows the observation of changes in regional cerebral blood flow by measuring changes in the concentration of cerebral haemoglobin (Hoshi, 2007). The sources were laser diodes emitting light at discrete wavelengths; 765 and 855 nm. So that the attenuation data could be converted into relative concentration changes of oxygenated haemoglobin (O2Hb), deoxygenated haemoglobin (HHb) and total haemoglobin (THb), a modified Beer-Lambert law[3] (Obrig and Villringer, 2003) was applied using the proprietal software, with the differential pathlength factor (the gradient of the attenuation with respect to the absorption coefficient of the tissue, used to determine the absolute pathlength of the photons, Kohl et al., 1998), adjusted according to the age of the participant. The resulting measurement is considered to express concentration changes in micromoles per litre (Toichi et al., 2004). Measurements were recorded at a time resolution of 10 Hz.

For this experiment, a 2-channel configuration was used (i.e. 2 emitter/optode pairs) with an emitter/optode separation distance of 40 mm. The emitter/optode pairs were positioned over the left and right frontal cortex using a standard optode holder headband (see Figure 5.2a), corresponding to points Fp1 and Fp2 in the 10-20 system. At this distance, the NIR signal is sensitive to hemodynamic changes within the top 2-3 mm of the cortex (Chance et al., 1988, see Figure 5.1). Since the same headband was used throughout the experiment, the relative positions of the emitter/optodes did not change from participant to participant. The emitter/optode pairs were attached to the Oxymon machine via 3 m long fibre optic bundles; the machine itself was connected via USB to a laptop for data acquisition (see Figure 5.2b). Recorded data were stored as a .TXT file and analysed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

---

[3] The Beer-Lambert law expresses a linear relationship between absorbance and concentration. A modified Beer-Lambert law is used for a highly scattering medium e.g. brain tissue.

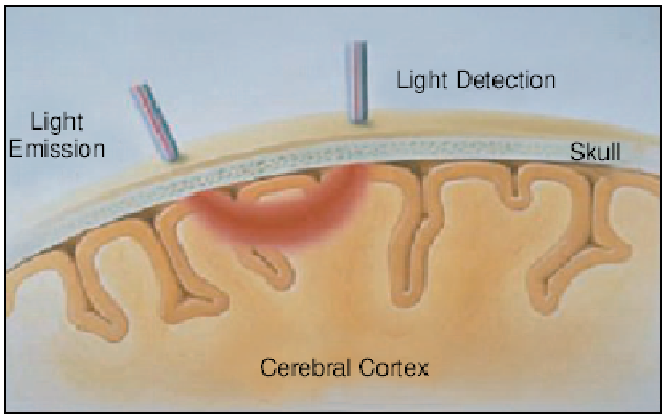

Figure 5.1. Photon path between NIRS emitter and detector pair. Reproduced from (Bunce et al., 2006).

a.

b.

Figure 5.2. Optode placement of the 2-channel NIRS headband (a). The optodes were connected via 3m long optic fibre bundles that plugged into the front of the NIRS device, where a dedicated laptop recorded the measurements (b).

In order to prevent artefacts due to changes in hydrostatic pressure, participants were instructed to remain in the same posture for the duration of each task, but were allowed to change their posture during the rest periods between each task and the task instruction pages. NIRS data were therefore time stamped by the researcher at the start and end of each task so that only NIRS data collected during the tasks were analysed.

5.2.5 Cognitive Tasks

Four tasks were selected that have previously been shown to activate the prefrontal cortex in brain imaging studies; Stroop Task (Vanderhasselt et al., 2009), Tower of London (Fitzgerald et al., 2008), 3-back task (Fitzgerald et al., 2008) and Wisconsin Card Sorting Task (Toone et al., 2000). Tasks were presented via a laptop computer in the same order as listed below. Progress through the battery of tasks was controlled by the participant, with brief instructions given on-screen prior to the start of each task. A 60-second rest period between each task was incorporated into the battery commencing as soon as the last response of the previous task had been made and ended with the presentation of the on-screen instructions for the next task. Data from these tasks were not analysed, but average scores can be found in Appendix VI.

5.2.5.1 Stroop Task

A computerised version of the Stroop task (Stroop, 1992) was presented. Words of colours (GREEN, BLUE, RED, YELLOW) were randomly presented in incongruently coloured text (e.g. GREEN was presented in blue text etc.). For each stimulus presentation, participants were instructed to press the coloured button on a response box that matched the colour of the text the word was presented in. This task was 5 minutes in duration. Overall accuracy (% correct) and reaction time were recorded.

5.2.5.2 Tower of London (TOL)

In this computerised version of the executive function task participants were presented with two configurations of three coloured balls (blue, green, red) on three pegs that hold three, two and one ball respectively. The subjects have to rearrange the balls from the starting configuration so that they match the position of the balls in the goal configuration. Subjects are asked to work out the entire planning sequence and indicate the minimum number of moves required prior to moving the balls using the mouse/cursor. The rules concerning the moving of the balls correspond to those of (Shallice, 1982): (1) only one ball could be moved, (2) a ball could only be moved if there was no ball above it on the peg and (3) the length of the pegs should be kept in mind. Subjects randomly complete 3 trials each which can be solved in 3, 4, and 5 moves respectively. Average planning time and time to complete the moves were recorded.

5.2.5.3 3-back Task

A continuous string of letters (upper and lower case; inter-stimulus interval of 2.5 seconds) was presented; 78 letters in total with 36 target pairs. For each stimulus, participants were instructed to indicate using a yes/no key press whether this was the same letter that appeared three letters previously. Average accuracy (% correct) and reaction time were recorded.

5.2.5.4 Wisconsin Card Sort Task (WCST)

This is a standard clinical measure that is commonly used as a measure of executive function and is thought to require cognitive flexibility, problem solving, and the use of feedback to guide behaviour. In this computerised version of the test, subjects are asked to match cards that vary by colour, shape, and number to four "key cards." Subjects are not told how to sort the cards, but must determine the correct category from the feedback given to their responses, which changes periodically throughout the test. In total the current version comprised the presentation of 128 cards. Overall accuracy (% correct) and time to complete the task were recorded.

5.2.6 Procedure

As part of a larger intervention trial investigating the effects of two types of fish oil on cognitive function and mood, participants had been taking a daily 1 g supplement of either EPA-rich FO, DHA-rich FO or olive oil placebo for 12 weeks. Towards the end of the 12 week period, participants were invited via email to take part in an extra cognitive testing session with concurrent NIRS data collection. In all cases this session was either on the day of the final behavioural testing session or the following day (average time on treatment 88.59 days). Testing took place in the afternoon (the cognitive assessments described in Chapter 4 took place in the morning), and participants were instructed to refrain from consuming food and drink except water for a minimum of 2 hours prior to testing.

On arrival participants were seated in front of the laptop computer used for presenting the cognitive tasks. They were fitted with the NIRS headband and were instructed to sit quietly with their eyes closed until they were given further instruction by the researcher to start the cognitive tasks. Recording of NIRS data was initiated

following 30 seconds of eyes-closed relaxation and the participant was instructed to begin the tasks after a further 2 minutes of eyes-closed relaxation. This 2 minute period was utilised as the NIRS resting baseline measurement. Participants completed each task as described above with NIRS data collected throughout.

5.2.6.1 Statistical methods

NIRS data were initially converted to 'change from baseline' (2 minute pre-testing rest period), the resultant data therefore representing the change in concentration of each of the chromophores with respect to the pre-testing measurements.

Prior to the primary analysis a within subjects Analysis of Variance (ANOVA) was carried out with left/right optode included as a factor (hemisphere x treatment group) for each task. As there were no treatment related interactions involving hemisphere the data from the 2 channels were averaged across hemispheres for the analysis and figures reported below.

The Stroop task was 5 minutes in length so NIRS data for this task was taken from 300 s of measurement. Due to the fact that the other three tasks were completed in variable lengths of time, the minimum duration of each task was identified and data for every participant were truncated accordingly. Therefore, data from TOL were averaged from 160 s of NIRS recording, for the 3-back task data were averaged over 250 s and for the WCST data were averaged over 180 s of NIRS recording. As the duration of each task of averaged NIRS data entered into the analysis was substantially longer than the potential physiological oscillations that can cause drift in shorter periods of NIRS recording (e.g. heartbeat, respiration Hoshi, 2007), no adjustment was required for this phenomenon (Kennedy et al., In press-b).

The primary analysis of the averaged NIRS data was conducted by one-way ANOVA for each task with *a priori* planned comparisons of the data from each task being made between placebo and each treatment group (DHA-rich FO, EPA-rich FO) using t tests calculated with the mean squares error of the overall ANOVA (Keppel, 1991). To protect against an increased Type I error rate, only those planned comparisons associated with a significant main effect of treatment on the ANOVA are reported.

5.3 Results

5.3.1 NIRS

5.3.1.1 Oxyhaemoglobin (O2Hb)

There was a significant main effect of treatment on O2Hb whilst participants completed the Stroop task [$F(2, 19) = 3.72$, $p = 0.043$]. Reference to the planned comparisons showed a significant increase in the concentration of O2Hb following the DHA-rich FO during this task compared with placebo [$t(19) = 2.73$, $p = 0.013$, $r = 0.59$]. Trends for an effect of treatment were observed for TOL and the 3-back task [$F(2, 19) = 3.35$, $p = 0.057$; $F(2, 19) = 2.69$, $p = 0.093$, respectively] (See Figure 5.3).

5.3.1.2 Deoxyhaemoglobin (HHb)

The ANOVAs revealed no treatment-related effects on concentrations of HHb during any of the cognitive tasks.

5.3.1.3 Total haemoglobin (THb)

The ANOVA showed that there was a significant effect of treatment on THb during the Stoop task, TOL and 3-back task [$F(2, 19) = 4.93$, $p = 0.019$; $F(2, 19) = 4.21$, $p = 0.031$; $F(2, 19) = 3.57$, $p = 0.048$, respectively]. Reference to the planned comparisons showed a significant increase in THb in the DHA-rich FO treatment condition compared to placebo during all three of these tasks [Stroop $t(19) = 3.10$, $p < 0.006$, $r = 0.64$; TOL $t(19) = 2.87$, $p < 0.009$, $r = 0.61$ and 3-back task $t(19) = 2.67$, $p < 0.016$, $r = 0.58$] (See Figure 5.3).

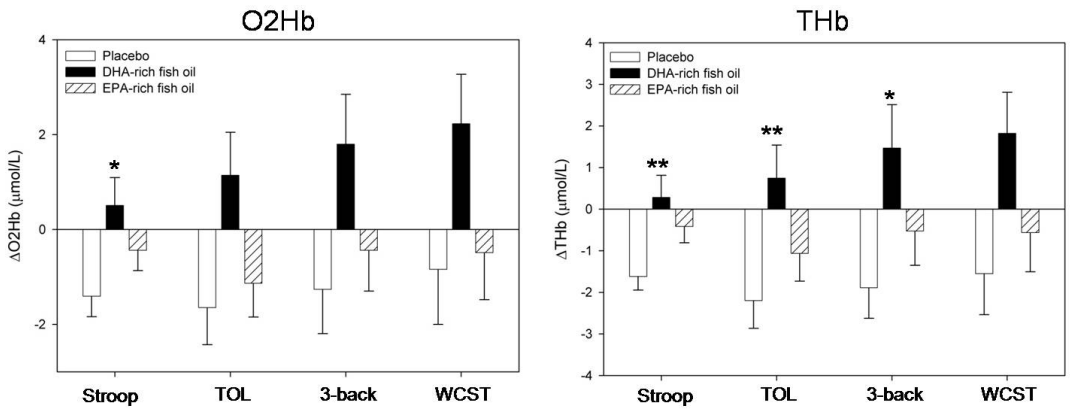

Figure 5.3. Concentration changes of oxygenated and total haemoglobin (Δ[O2Hb] and Δ[THb]) by treatment group (Placebo, DHA-rich FO, EPA-rich FO) during the cognitive tasks. Data are averaged across both hemispheres (left/right). Significance is compared to placebo (planned comparisons). (*, $p < 0.05$; **, $p < 0.01$).

5.4 Discussion

The results of the current study revealed that participants administered 1 g/d DHA-rich FO for 12 weeks had an increased CBF response to performing cognitive tasks in the prefrontal cortex, in comparison with placebo, as indexed by changes in the concentration of total haemoglobin (THb) and oxyhaemobglobin (O2Hb). A significant difference between the placebo and DHA-rich FO treatment groups on either of these chromophores was not universally observed, however. Reference to the overall pattern of concentration changes during all the tasks for both these chromophores suggests perhaps that the small sample size did not allow for adequate detection of a difference in the concentration changes in the DHA-rich FO treatment group compared to placebo during the other tasks. With regards to concentration changes of deoxyhaemoglobin (HHb), no difference was observed between either of the fish oil treatment groups and placebo during any of the tasks, though change from baseline concentrations were similarly small in all three conditions, a phenomenon that has been observed previously (Hoshi and Tamura, 1993). In addition, no significant effects of treatment were observed on any of the cognitive performance measures.

The pattern of concentration changes in O2Hb and THb in the EPA-rich FO group did not differ from placebo which suggests that the higher daily dose of DHA in the DHA-rich FO (450 mg/d compared to 200 mg/d) contributed to the observed concentration changes. More specifically, this finding suggests that rCBF response to tasks is only modulated following supplementation with DHA at a dose that is higher than 200 mg/d. Also, the pattern of increased O2Hb and THb in the DHA-rich FO treatment group, compared to placebo, is comparable to data published from a supplementation study in aged monkeys, in which DHA (150 mg/kg/d) was administered to the animals for 4 weeks. The results of this study showed that compared to placebo, the age-related impairment of the rCBF response to tactile stimulation (as measured by PET) was restored (Tsukada et al., 2000b), again providing evidence supporting a role for DHA in cerebrovascular function. On the other hand, the data presented here suggest that despite the fact that EPA is known to improve peripheral cardiovascular parameters, the addition of dietary EPA at a dose of 300 mg/d for 12 weeks has no impact on cerebral hemodynamic response to cognitive tasks.

Overall the results of this pilot study therefore provide the first indication of possible modulation of cerebral blood flow parameters following dietary supplementation with DHA-rich FO, and at a dose (1 g/d) that would be achievable by dietary intake of oily fish alone. Previously, studies that have aimed to evaluate the effect of n-3 PUFA supplementation on behavioural outcomes in healthy individuals have provided mixed results, but the data from the current study suggest that an n-3 PUFA dietary supplement may indeed impact brain physiology as regards cerebral perfusion of activated areas. Given these interesting and novel results, further studies using NIRS to assess the causal relationship between n-3 PUFA intake and cerebral blood flow parameters are warranted.

CHAPTER 6. THE COGNITIVE AND CEREBRAL HEMODYNAMIC EFFECTS OF DHA-RICH FISH OIL: A DOSE-RANGING STUDY

6.1 Introduction

The n-3 PUFAs DHA and EPA are vital for normal cell function; however the distribution of these fatty acids is not uniform across bodily organs. For example, 10-20% of the total fatty acids in the brain are comprised of DHA, yet less than 1% are comprised of EPA (McNamara and Carlson, 2006). The DHA composition of the brain (and indeed the DHA and EPA content of other tissues) is incredibly labile, owing to the fact that DHA, along with the other n-3 PUFAs, must be acquired via dietary sources. Rats and non-human primates raised on diets deficient in n-3 PUFAs (DHA, EPA and their parent precursor fatty acid ALA) have reduced levels of DHA in the brain, which is associated with modified cholinergic, serotonergic and dopaminergic neurotransmission pathways (Delion et al., 1994, Aid et al., 2003). In line with these physiological changes, reduced n-3 intake has also been shown to be associated with behavioural changes. For example, rats showed reduced learning on the Morris Water Maze task as evidenced by delayed acquisition and longer escape latencies (Moriguchi et al., 2000, Jensen et al., 1996) and poorer performance on olfactory discrimination tasks (Greiner et al., 2001). Similarly, rats raised on n-3 deficient diets showed more working memory and reference memory errors on the radial arm maze task, compared to those that were brought up on the same n-3 deficient diet and then supplemented with dietary DHA (Gamoh et al., 1999, see also Section 1.5.1).

A similar relationship between dietary n-3 PUFA intake, biological markers and behaviour appears to exist in humans, though it is more difficult to separate the impact of individual n-3 PUFAs. For example, the plasma n-3 PUFA profiles of consumers of oily fish have higher concentrations of DHA, EPA and ALA than non-consumers (Welch et al., 2006), and grey matter volume in healthy adults is positively associated with total n-3 PUFA intake, estimated by 24-hour food recall (Conklin et al., 2007). In terms of behaviour, low maternal intake of oily fish during pregnancy is associated with increased risk for suboptimal cognitive, developmental and behavioural outcomes in later childhood (Hibbeln et al., 2007). Further, in

comparison with normal healthy individuals, those diagnosed with developmental disorders such as autism and ADHD (Bell et al., 2000, Burgess et al., 2000, Schuchardt et al., 2009) or certain neuropsychiatric illnesses including depression (Edwards et al., 1998), schizophrenia (Assies et al., 2001) and Alzheimer's disease (Conquer et al., 2000) have lower tissue (plasma/serum, erythrocyte membrane, adipose tissue) concentrations of n-3 PUFAs, however intervention trials to investigate the potential for n-3 PUFAs in the treatment of these conditions have yielded mixed results (see Section 1.5.2.1). N-3 PUFA supplementation for cognitive and mood enhancement has also recently been explored in healthy volunteers, but again these supplementation studies have not always shown benefits of the intervention over placebo on behavioural outcomes (see Sections 1.5.2.3, 2.3, 4.3). However, despite the lack of evidence to support the use of n-3 PUFAs for improved behavioural outcomes, it is plausible to suggest that the addition of dietary n-3 PUFAs may still be having an impact on brain function. It is possible that the lack of consistency in the behavioural data may be an artefact of methodological diversity (e.g. sample, dose and treatment formulation, time on treatment, outcome measures) between studies, or simply that the effects of n-3 PUFAs on physiological function in the brain or elsewhere in the body are not large enough to impact directly on behaviour.

In the pilot study described in Chapter 5, the effects of either a DHA-rich fish oil (FO; 450 mg DHA + 90 mg EPA) or EPA-rich FO (300 mg EPA + 200 mg DHA) on the regional cerebral blood flow response to task performance was investigated using Near Infrared Spectroscopy (NIRS) in healthy human adults. It was demonstrated that, compared to placebo, participants administered the DHA-rich FO had increased concentrations of total haemoglobin (THb) and oxyhaemoglobin (O2Hb) in the prefrontal cortex when responding to a selection of cognitive tasks (see Section 5.3 for details). Whilst this pilot study had low statistical power, the results for the DHA-rich FO treatment were promising enough to take forward into a larger trial which would have the added advantage of providing a more sufficient assessment of treatment effects on task performance. The present study aimed to address this issue, with a view to evaluating the effects of treatment on brain function (cerebral hemodynamics) and behaviour (cognitive performance) in parallel. Previous intervention studies in n-3 deficient rats have suggested that supplementing the diet with n-3 PUFAs to excess does not result in any additional positive effects on performance (Farkas et al., 2002), but the impact of n-3 PUFAs

on cerebral blood flow at levels higher than that which would easily be achievable by dietary means alone is not known. For this reason two doses of DHA-rich FO were selected for evaluation in the present study; 1 g/d is roughly equivalent to consuming one portion of oily fish per week (450 mg n-3 PUFAs), the minimum intake recommended by the current advisory on fish consumption (SACN/COT, 2004), and 2 g/d, which would be equivalent to twice this amount and more difficult to achieve through dietary sources alone. Further, non-consumers of oily fish were invited to participate in the study in order to control for dietary intake of DHA during the study period, given that oily fish is the most abundant dietary source of DHA. Despite opting to select a sample according to this particular criterion, it could also be argued that recruiting only non-consumers of oily fish is still representative of the large proportion of the general population, given that in the last National Diet and Nutrition Survey in 2002 it was revealed that 74% of adults aged 19-64 years do not consume oily fish (Henderson et al., 2002). Therefore the current study aimed to investigate the effects of two doses of DHA-rich FO on task-related cerebral hemodynamic response and cognitive performance in healthy non-consumers of oily fish.

6.2 Materials and Methods

6.2.1 Design

A placebo-controlled, double-blind independent measures design was employed with participants randomly assigned to one of three treatment groups (placebo, 1 g DHA-rich fish oil, 2 g DHA-rich fish oil; see Section 6.2.3). Prior to the start of the study a restricted randomisation (23 X 3 treatments) list matching treatments to participant code numbers was computer generated. Participants were assigned the next available code at the screening session. For the NIRS data analysis, task comprised a second, within subjects factor.

6.2.2 Participants

Sixy-five healthy adults (mean age 20.58 years, range 18-29) were recruited via an email advert sent to Northumbria University undergraduate students. All participants declared they were in good health, a non-smoker, free from prescription medication and social drugs, free from omega-3 supplements and a native English speaker. They also declared that they were non consumers of oily fish. One participant withdrew from the study due to illness; demographic details for the remaining 64 participants who completed the study can be found in Table 6.1.

The study received approval from the Northumbria University School of Psychology and Sport Sciences Ethics Committee and was conducted according to the Declaration of Helsinki (1964). All participants gave their informed consent prior to their inclusion in the study.

Table 6.1. Demographic data by treatment group. Means are presented with SEM.

	Placebo		1 g fish oil		2 g fish oil	
	Mean	SEM	Mean	SEM	Mean	SEM
N (M/F)	6/14		7/15		3/19	
Age	21.35	0.62	20.50	0.43	19.95	0.34
BMI	23.73	0.88	22.23	0.63	22.37	0.76

6.2.3 Treatments

Participants received two containers of 196 capsules labelled Container 1 and Container 2, along with instructions to take 2 x 500 mg capsules from each container every day for the duration of the experiment (a daily total of 4 x 500 mg capsules for 12 weeks). For the placebo group Container 1 and Container 2 both held olive oil capsules, for the 1 g FO group Container 1 held active capsules and Container 2 held olive oil capsules and for the 2 g FO group both containers held active capsules. The DHA-rich FO and placebo capsules were identical to those described in Section 4.2.3; each of the active capsules contained 497.5 mg of deodorised FO plus 2.5 mg mixed tocopherols and each of the placebo capsules contained 500 mg olive oil. The total daily dose of n-3 PUFAs for the 1 g FO group was 450 mg DHA + 90 mg EPA and for the 2 g FO group these amounts were 900 mg DHA + 180 mg EPA.

Prior to the start of the study a restricted randomisation (30 X 3 treatments) list matching treatments to participant code numbers was computer generated. Participants were then assigned a participant code and the corresponding treatment at the end of the screening visit. All treatments were packaged, labelled and randomised on site by a disinterested third party who had no further involvement in any aspect of the study.

6.2.4 Near Infrared Spectroscopy

The study utilsed a 2-channel continuous wave Oxymon system described previously in Chapter 5.2.3.1. In this system the emitter/optode pairs were positioned over the left and right frontal cortex using a standard optode holder headband, corresponding to points Fp1 and Fp2 in the 10-20 system. NIRS data were time stamped by the researcher at the start and end of each task so that only NIRS data collected during the tasks were analysed.

6.2.5 Cognitive Assessment

Nine tasks were administered using the COMPASS (Computerised Mental Performance Assessment) system, which presented a battery of standard cognitive

tasks in the same order as outlined below (cognitive domain in brackets)**.** For the purposes of the behavioural analysis, the tasks were selected with the intention that performance on a range of cognitive functions could be assessed including working memory (WM), attention and executive function. Specifically, they were also selected on the basis that execution of the same or a similar task had shown activation in the frontal cortex in previous imaging studies (although it is assumed that activation is not exclusively limited to this region), given that this was the region of interest in the current study [Corsi Blocks (Owen et al., 1996); NWM (Awh et al., 1996); 3-back (Fitzgerald et al., 2008); SRT, 4CRT (Rosenthal et al., 2001); CRT (Schluter et al., 2001); Stroop (Vanderhasselt et al., 2009); RVIP (Lawrence et al., 2002); Serial 7s (Drummond et al., 1999)]. Progress through the battery of tasks was controlled by the participant, with brief instructions given on-screen prior to the start of each task.

6.2.5.1 Corsi Blocks Task (Spatial WM)

In this task nine identical blue squares appeared on screen in non-overlapping random positions. A set number of blocks changed colour from blue to red in a randomly generated sequence. The cursor was locked in position until the entire sequence had been presented, at which point the participants were instructed to repeat the sequence by clicking on the blocks using the mouse and cursor. The task was repeated five times at each level of difficulty. The sequence span increased from 4 upwards, until the participant can no longer correctly recall the sequence, resulting in a span measure of nonverbal working memory, calculated by averaging the level of the last five correctly completed trials.

6.2.5.2 Numeric Working Memory (NWM)

Five random digits from 1-9 were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits (15 targets and 15 distractors) for each of which the participant indicated whether or not it had been in the original series by a simple key press. The task consisted of 3 separate trials. Accuracy and mean reaction time were recorded.

6.2.5.3 3-back Task (WM)

A continuous string of letters (upper and lower case; inter-stimulus interval of 2.5 seconds) was presented; 45 letters in total with 15 target pairs. For each stimulus, participants were instructed to indicate whether this was the same letter that appeared three letters before. Accuracy and mean reaction time were recorded.

6.2.5.4 Simple Reaction Time (SRT – psychomotor speed/attention)

The participant was instructed to press the 'space bar' on the laptop keyboard as quickly as possible every time an upwards pointing arrow appeared on screen. Sixty-four stimuli were presented with an inter-stimulus duration that varied randomly between 1 and 3.5 seconds. Mean reaction time was recorded.

6.2.5.5 Choice Reaction Time (CRT – attention/response inhibition)

An arrow appeared on the screen pointing to the left or to the right. Participants responded with a left or right key press corresponding to the direction of the arrow. Sixty stimuli were presented with a randomly varying inter-stimulus interval of between 1 and 3 seconds. Accuracy (% correct) and mean reaction time were recorded.

6.2.5.6 Four Choice Reaction Time (4CRT - attention)

A visual representation of the four direction arrow keys of a standard keyboard was presented on screen. The arrows 'lit up' at random on screen until the corresponding key press was made. In all, each arrow was the target stimulus 15 times, forming a total of 48 stimuli for this task in all. Accuracy and mean reaction time were recorded.

6.2.5.7 Stroop Task (attention/response inhibition)

A computerised version of the Stroop task (Stroop, 1992) was used. Words describing colours (GREEN, BLUE, RED, YELLOW) were randomly presented in either congruently or incongruently coloured text (e.g. GREEN was presented in blue text etc.). For each stimulus presentation during this 2 minute task, participants were instructed to use the computer mouse and cursor to click the colour box located on the right side of the screen that matched the colour of the text the word was presented in. Accuracy and mean reaction time were recorded.

6.2.5.8 Serial 7 subtractions (Serial 7s – WM)

In this two-minute task, participants were instructed to count backwards in 7s as quickly and as accurately as possible using the computer keyboard's linear number keys to enter each response. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each subsequent 3-digit response was represented on screen by an asterisk. Total number of subtractions and errors were calculated.

6.2.5.9 Rapid Visual Information Processing task (RVIP – sustained attention)

During this 4 minute task the participants monitored a continuous series of single digits (1-9) for targets of three consecutive odd or three consecutive even digits. The digits were presented on the computer screen at the rate of 100 per minute in a pseudo-random order, with the participant responding to the detection of a target string with a space bar key press. Eight target strings are presented in each minute. The task is scored for number of correctly identified target strings, average reaction time for correct detections and number of false alarms.

6.2.6 Procedure

Each participant was required to attend the laboratory on three occasions. The first of these was an initial screening visit, in which participants provided written informed consent and were screened with regards to the study exclusion/inclusion criteria. Demographic data were recorded, and particpants were randomly allocated to a treatment group. They were given the two containers of capsules along with written instructions for taking the study treatment and a diary card, which they were instructed to complete every day that the capsules were taken. A training session comprised the participant's second visit to the laboratory, which took place in the afternoon approximately 7 days before the final testing session. During this visit participants completed the battery of cognitive tasks once, without concurrent NIRS recording, in order to familiarise them with the demands of each task. Participants were trained simultaneously in groups of no more than 6, but were visually isolated from each other throughout the session.

The final testing session took place after participants had been taking the study treatment for 12 weeks (average time on treatment 87.08 days). On this occasion, participants were tested individually, with concurrent NIRS recording. They attended the laboratory in the morning in an overnight fasted state, having consumed no food or drink except water for 12 hours prior to testing. On arrival participants were fitted with the NIRS headband; as soon as NIRS data capture was initiated participants were instructed to close their eyes and relax for a duration of 5 minutes, with the last minute of this period utilised as the NIRS resting baseline measurement. They were then verbally instructed to start the battery of tasks as described above with NIRS data collected throughout.

6.2.6.1 Statistical methods

NIRS data were converted to 'change from baseline' (1 minute pre-testing rest period) and averaged across each task during the cognitive task performance period. The resultant data therefore represented the change in concentration of each of the chromophores with respect to the pre-testing measurements.

Task length was fixed for the Stroop (120 s), RVIP (240 s) and Serial 7s (120 s) tasks, but NIRS data from the remaining six tasks were truncated so that the same amount of data was analysed from all participants during each task period. For the Corsi blocks task only data from the first 210 s of testing was used, for NWM the first 120 s of data were used, for the 3-back task the first 150 s were used and the first 160 s were used for SRT, CRT and 4 CRT. As the duration of each task of averaged NIRS data entered into the analysis was substantially longer than the potential physiological oscillations that can cause drift in shorter periods of NIRS recording (e.g. heartbeat, respiration - Hoshi, 2007), no adjustment was required for this phenomenon.

Prior to the primary analysis a mixed Analysis of Variance was carried out with right/left optode included as a factor [hemisphere x treatment group x task] to examine any hemispheric differences in response. As there were no treatment related interactions involving this factor the data from the two channels were averaged across hemispheres for the analysis and figures reported below.

The primary analysis of the averaged NIRS data was conducted by mixed ANOVA (treatment group x task) for each chromophore (O2Hb, HHb, THb) with *a priori* planned comparisons of data from each task period being made between placebo and each of the fish oil treatment groups (1 g, 2 g) using t tests calculated with the mean squares error utilised from the omnibus ANOVA (Keppel, 1991). In order to reduce the potential for Type I errors only those planned comparisons associated with a significant main effect of treatment or interaction between treatment and task, revealed in the ANOVA, are reported.

The performance data were analysed by between-subjects ANOVA for each individual task outcome with planned comparisons for data as described above.

6.3 Results

6.3.1 Compliance

Compliance was assessed using participants' self-report diary cards to determine on how many days the capsules had been taken along with the number of capsules returned on the day of the final testing session; 1 participant failed to return their unused capsules and diary card. For the remaining participants, compliance was very good in all three treatment groups (96% placebo, 91% 1 g FO, 92% 2 g FO). At the very end of the final testing session participants were also asked to guess which treatment they had taken (FO or placebo). Participants who had received the DHA-rich FO (either 1 g or 2 g) were equally as likely to guess correctly (52%) as those in the placebo group (50%), at or about chance level [χ^2 (1) = 1.52, p = 0.22], indicating a successful double-blind procedure.

6.3.2 NIRS

6.3.2.1 Oxyhaemoglobin (O2Hb)

The ANOVA showed that there was a significant main effect of treatment [$F(2, 472) = 3.54$, $p = 0.035$]. Planned comparisons of the overall treatment means revealed significantly increased concentrations of O2Hb in both the 1 g and 2 g FO groups [$t(472) = 3.79$, $p = 0.0002$, $r = 0.52$; $t(472) = 4.71$, $p < 0.0001$, $r = 0.60$]. In addition, reference to the planned comparisons carried out on data from each task period showed that both 1 g and 2 g FO were associated with a significantly (all $p < 0.0001$, see Figure 6.1) increased concentration of O2Hb during the performance of each task.

6.3.2.2 Deoxyhaemoglobin (HHb)

The ANOVA did not reveal a significant effect of treatment on concentrations of HHb.

6.3.2.3 Total haemoglobin (THb)

The ANOVA showed that there was a significant effect of treatment [$F(2, 472) = 3.67$, $p = 0.032$]. Planned comparisons of the overall treatment means revealed significantly increased concentrations of O2Hb in both the 1 g and 2 g FO groups [$t(472) = 3.51$, $p = 0.0005$, $r = 0.49$; $t(472) = 4.64$, $p < 0.0001$, $r = 0.60$]. The planned comparisons carried out on data from each task period showed that 2 g FO was associated with a significantly (all $p < 0.001$) increased concentration of THb during the performance of each task. The 1 g FO treatment was also associated with significantly increased concentration of THb during each task period, although these associations were not as highly significant (all $p < 0.05$). These data are also presented in Figure 6.1.

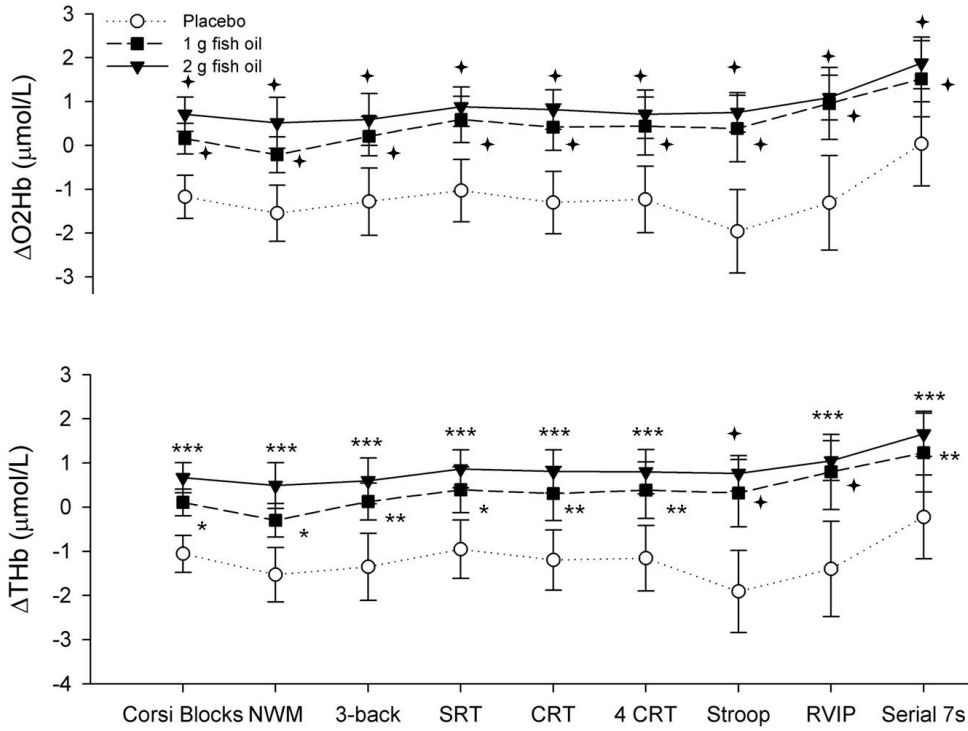

Figure 6.1. Concentration changes of oxygenated and total haemoglobin (Δ[O2Hb] and Δ[THb]) by treatment group (Placebo, 1 g fish oil, 2 g fish oil) during the cognitive tasks. Data are averaged across both hemispheres (left/right). Significance is compared to placebo (planned comparisons). (*, $p < 0.05$; **, $p < 0.01$ ***, $p < 0.001$; ✦, $p < 0.0001$).

6.3.3 Cognitive Performance

There was a significant main effect of treatment on reaction time for the CRT task [$F(2, 61) = 3.27$, $p = 0.045$]. Planned comparisons revealed that reaction time on

this task was faster in both treatment groups (1 g FO [$t(61) = 2.67$, $p = 0.027$, $r = 0.34$], 2 g FO [$t(61) = 2.20$, $p = 0.032$, $r = 0.33$]). There was also a significant main effect of treatment on the RVIP task [$F(2, 61) = 6.75$, $p = 0.002$]. Reference to the planned comparisons revealed that reaction time was significanly faster in the 2 g FO group, compared to placebo [$t(61) = 2.90$, $p = 0.005$, $r = 0.42$]. Performance data for all tasks is displayed in Table 6.3.

Table 6.2. Cognitive task performance by treatment group. Means and SEM are presented with *F* and *p* values from the primary ANOVA. Significance is indicated in bold typeface.

		Mean	SEM	*F*	*p*
Corsi blocks Span	Placebo	5.72	0.06		
	1 g fish oil	5.61	0.07	2.52	0.089
	2 g fish oil	5.45	0.12		
NWM (% accuracy)	Placebo	96.28	0.83		
	1 g fish oil	96.52	0.89	0.44	0.646
	2 g fish oil	95.51	0.68		
NWM (RT)	Placebo	498.27	22.03		
	1 g fish oil	509.86	18.99	0.08	0.927
	2 g fish oil	508.12	25.59		
3-back (% accuracy)	Placebo	90.00	2.09		
	1 g fish oil	91.62	1.29	0.60	0.551
	2 g fish oil	89.09	1.62		
3-back (RT)	Placebo	1207.44	104.80		
	1 g fish oil	1315.76	104.39	1.56	0.219
	2 g fish oil	1060.13	103.79		
SRT	Placebo	316.41	13.74		
	1 g fish oil	288.80	9.52	1.42	0.249
	2 g fish oil	304.77	11.39		
CRT (% accuracy)	Placebo	97.72	0.70		
	1 g fish oil	95.74	0.80	1.99	0.146
	2 g fish oil	96.53	0.57		
CRT (RT)	Placebo	423.18	14.96		
	1 g fish oil	389.02	8.07	**3.27**	**0.045**
	2 g fish oil	390.02	8.09		
4 CRT (% accuracy)	Placebo	98.74	0.42		
	1 g fish oil	98.54	0.32	0.11	0.901
	2 g fish oil	98.43	0.62		
4 CRT (RT)	Placebo	498.46	25.47		
	1 g fish oil	471.37	12.92	0.64	0.533
	2 g fish oil	487.01	10.48		
Stroop congruent (% accuracy)	Placebo	98.34	0.60		
	1 g fish oil	97.90	0.68	0.17	0.841
	2 g fish oil	97.82	0.66		
Stroop congruent (RT)	Placebo	651.10	23.72		
	1 g fish oil	626.91	22.78	0.56	0.573
	2 g fish oil	619.00	19.47		
Stroop incongruent (% accuracy)	Placebo	97.30	0.79		
	1 g fish oil	96.96	0.65	0.75	0.476
	2 g fish oil	98.02	0.44		
Stroop incongruent (RT)	Placebo	706.60	28.82		
	1 g fish oil	671.73	24.01	0.84	0.436
	2 g fish oil	665.55	18.12		

		Mean	SEM	*F*	*p*
Stroop overall (% accuracy)	Placebo	97.64	0.68		
	1 g fish oil	97.29	0.52	0.36	0.700
	2 g fish oil	97.93	0.41		
Stroop overall (RT)	Placebo	687.20	26.11		
	1 g fish oil	655.73	23.11	0.81	0.450
	2 g fish oil	649.14	17.30		
RVIP (% accuracy)	Placebo	59.22	5.03		
	1 g fish oil	58.38	4.55	1.30	0.281
	2 g fish oil	67.14	3.19		
RVIP (RT)	Placebo	479.27	19.34		
	1 g fish oil	487.05	8.20	**6.75**	**0.002**
	2 g fish oil	422.78	12.23		
RVIP (false alarms)	Placebo	13.05	1.61		
	1 g fish oil	13.32	1.46	1.31	0.277
	2 g fish oil	10.50	1.02		
Serial 7s (no. correct)	Placebo	20.20	2.26		
	1 g fish oil	24.68	3.22	0.64	0.531
	2 g fish oil	23.45	2.89		

6.4 Discussion

The primary aim of the current study was to investigate the effects of 12 weeks supplementation with 1 g/d or 2 g/d DHA-rich FO on task-related cerebral hemodynamic response in non-consumers of oily fish. Supplementation with DHA-rich FO was associated with a pattern of significantly increased regional cerebral blood flow (rCBF) in the prefrontal cortex, in comparison with placebo, as indexed by changes in the concentration of total haemoglobin (THb) and oxyhaemoglobin (O2Hb) during the cognitive tasks. In contrast, there was no evidence of modulation of deoxyhaemoglobin (HHb) by either dose of DHA-rich FO compared with placebo. Overall these patterns of task-related hemodynamic response of both active treatments and placebo are consistent with those observed in the pilot study described in Chapter 5. In addition, two main effects of treatment were detected as regards the cognitive tasks; participants in both treatment groups were faster than placebo on the Choice Reaction Time (CRT) task, and those in the 2 g FO group were quicker to respond on the RVIP task.

Given the lack of evidence of modulation of cerebral hemodynamics by the EPA-rich FO treatment (300 mg EPA + 200 mg DHA) in the pilot trial described in Chapter 5, it is reasonable to attribute the observed modulation of cerebral hemodynamics in the current study to the DHA contained in the FO formulation and not the relatively small quantity of EPA (90 mg/g DHA-rich FO). Indeed, the actions of DHA in the (cerebro)vascular system are numerous and complex, and evidence from *in vitro* investigations suggests that DHA could be acting on these parameters in a number of different ways. DHA may facilitate the cerebrovascular coupling mechanism via modulation of cholinergic neuronal transmission (Tsukada et al., 2000a, Tsukada et al., 1997), possibly as a result of increased cerebral acetylcholine levels following dietary intake of DHA (Minami et al., 1997). Even the incorporation of DHA into endothelial cell membranes may beneficially impact cerebrovascular response by promoting endothelial fluidity and improving membrane-bound protein function (Hashimoto et al., 1999). In addition, DHA has been shown to modulate the production of nitric oxide (Li et al., 2008), a potent second messenger that regulates the cerebral blood flow response in the brain (Kitaura et al., 2007). *In vivo* studies have also highlighted the effects of DHA on physiological functions in the brain. For example, a recent study in rats discovered dietary supplementation with DHA-rich FO (DHA 4:1 EPA) resulted in increased brain nitric oxide synthase activity

(Engstrom et al., 2009). Interestingly, in the same experiment EPA-rich FO (EPA 3:2 DHA) did not have any effect, reflecting the results reported in Chapter 5 in which cerebral hemodynamic response to tasks did not differ from placebo in the EPA-rich FO (EPA 3:2 DHA) treatment group. In addition, the same pattern of observed differences between placebo and DHA-rich FO treatment groups regarding cerebral hemodynamics was consistently detected in both the pilot trial described in the previous chapter (consumers; mean intake 2.05 portions/month) and the current study (non-consumers); also lending support to the assertion that DHA does indeed have an effect on cerebral hemodynamic response to tasks.

Turning to the cognitive performance measures that were employed, a significant difference between treatment groups was revealed on two of the tasks; participants in both treatment groups (1 g, 2 g) were faster than placebo on the Choice Reaction Time (CRT) task and those in the 2 g treatment group were faster on the RVIP task. Success on these two tasks requires response inhibition (CRT) and sustained attention (RVIP). In light of the results presented in Chapter 4, in which it was revealed that 12 weeks supplementation with 1 g/d DHA-rich FO (identical formulation to the DHA-rich FO utilised in the current study) reduced reaction time on the Stroop task compared with placebo, it is interesting that this effect was not replicated here. One possibility could be that the difference in response modality—the current study utilised a four-colour response box whereas the study described in Chapter 4 utilised the mouse and cursor—could account for the difference in findings between the two studies. The version chosen for the current study was selected on the premise that responding to the stimuli requires less movement using a button box than a mouse therefore reducing the possibility of motion artefacts. Of course another possibility is that the current study simply lacked sufficient power to detect an effect on this task; reference to the means presented in Table 6.3 do reveal a pattern of reduced reaction time on this task in the active treatment groups, compared to placebo.

In a similar vein, in light of the current study's results the fact that no effects of DHA-rich FO were observed on the CRT and RVIP tasks in the study described in Chapter 4 could be attributed to a number of factors. Firstly, the presentation of the tasks may be one factor, as this varied between studies. For example, the task length for the CRT and RVIP tasks utilised in the current study were longer and shorter respectively than in Chapter 4. The RVIP task in particular was employed in

Chapter 4 as part of the Cognitive Demand Battery, and due to the taxing nature of this battery there was evidence to suggest that participants were inclined to disengage with the task; as evidenced by the reduced number of datasets that were analysed for these outcomes (Table 4.4). It is also possible that performance on the tasks described in the current study might have been affected by the concurrent NIRS recording. Finally, the choice of a sample of non-consumers of oily fish is another consideration. Healthy non-consumers were selected in an attempt to control for past intake of DHA (and EPA) and also to control for intake during the intervention period with the aim of recruiting of a largely homogenous sample, from which stronger conclusions about the effects of the intervention on cerebral hemodynamics could be made. One possibility is that consumers and non-consumers of oily fish may perform differently on cognitive measures, although this has yet to be directly investigated in humans. In addition, the tenacity with which the brain retains DHA in particular—the half life of DHA in the brain is estimated at 2.5 years (Umhau et al., 2009)—could potentially explain why behavioural effects are not consistently observed in healthy individuals who consume even just the occasional portion of n-3 fatty acid-rich oily fish. Altogether these factors indicate that direct comparison of participants' performance on the tasks described in Chapters 4 and 6 should not be made. However, it is interesting that in both studies reaction time was reduced on attention tasks, perhaps representing an emerging pattern of an effect of DHA-rich FO on attention task performance. However, the results of the present chapter, taken as a study in and of itself, are in keeping with previous behavioural studies that have failed to show consistent effects of n-3 PUFAs in healthy populations. Even in this sample of adults who do not eat any oily fish (and presumably have low intake of dietary DHA and EPA), the results from the cognitive performance outcomes described here provide little evidence of cognitive enhancement following n-3 PUFA supplementation in healthy, cognitively intact individuals.

Considering the behavioural data in conjunction with the NIRS data, the findings of the current study reveal that DHA-rich FO is impacting brain physiological function—as evidenced by the difference between hemodynamic response to tasks in the active treatment groups and placebo—but that this is not consistently accompanied by improved task performance. One possibility is that administration of DHA is improving the overall function and health of the brain, but that this is not manifested in observable behavioural measures in a healthy young population. However,

increased cerebral blood flow might be a potential mechanism by which n-3 PUFAs derived from fish oils exert a positive effect on cognitive function in later life, as epidemiological studies have linked reduced cognitive decline and incidence of Alzheimer's disease in older adults with increased intake of fish and n-3 PUFAs (Morris et al., 2003, Dullemeijer et al., 2007, Kalmijn et al., 1997a, Engelhart et al., 2002). Indeed, reduced cerebral blood flow has been consistently observed in both normally ageing and demented older adults (Farkas and Luiten, 2001), and in animal studies reduced cerebral blood flow is associated with both neurodegeneration in the hippocampus and cerebral cortex as well as spatial memory deficits (Farkas et al., 2002). One hypothesis is that increased cerebral blood flow to the brain throughout the entire lifespan may protect against neural damage that occurs as part of the natural decline in cerebrovascular sufficiency (Farkas and Luiten, 2001). Research in monkeys demonstrated that age-impaired cerebral blood flow is indeed modulated by DHA; 4 weeks dietary supplementation of this fatty acid (150 mg/kg/d) facilitated the cerebral blood flow response to tactile stimulation (Tsukada et al., 2000a). Based on this group's previous findings (Tsukada et al., 1997), the authors suggest that the observed modulation of rCBF is not due to changes in global CBF, but rather could be attributed to changes in the coupling mechanism regulated by the cholinergic system. Given the above, an interesting avenue to pursue would be to investigate the cerebral hemodynamic and effects of DHA in elderly human participants.

As regards the methodology employed in the current study, a number of issues should be addressed. The first of these is the absence of pre-treatment baseline measures of cognitive performance and cerebral hemodynamics. As regards the cognitive performance measures, although it is assumed that random allocation would eliminate the possibility of systematic differences between treatment groups, further reduced by the fact that the participants were drawn from the same population (healthy university students), it is also widely accepted that adjusting for pre-treatment performance increases the power of the post-treatment between group analysis of variance (Van Breukelen, 2006), and would be a feature of future studies. However, due to the fact that NIRS instruments can only provide data regarding the relative concentration changes in cerebral hemodynamic parameters in response to tasks (taken from an arbitrary measure i.e. the point at which the machine was turned on), and does not provide any data regarding actual quantities of the chromophores, an ANCOVA of the data collected on the last day of the

intervention, using baseline NIRS measures as a covariate would not be possible. However, what a pre-treatment baseline measure of cerebral hemodynamics could establish is the absence of between-group differences in hemodynamic response to the tasks. This improvement to the paradigm could be used in future applications of NIRS in pharmacological interventions of this kind. In relation to this, a second improvement would be the addition of a measure of blood flow velocity to the brain. As previously, mentioned, the data collected by NIRS only reflects relative task-related concentration changes of the individual chromophores, and does not allow for predictions to be made about the effects of treatment on overall cerebral blood flow. Transcranial Doppler ultrasound follows the same principles and assumptions of other applications of the Doppler effect to measure blood flow velocity in the main intracranial vessels non-invasively, and with high accuracy. This measurement reflects CBF in the brain regions supplied by the vessel under investigation (Deppe et al., 2004, Panerai, 2009). Therefore, the addition of TCD in future investigations would allow for quantifiable changes in general cerebral blood flow as well as during the cognitive tasks following dietary intake of DHA to be calculated.

Also of note is the pattern of changes in concentrations of O2Hb and THb in the placebo treatment group, which fell below the resting baseline measurement during the tasks. This pattern of task-related concentration changes would not be expected given previous NIRS investigations (e.g. Fallgatter and Strik, 1998, Richter et al., 2009), but may simply reflect either the level of task difficulty, or a general pattern of falling CBF as arousal levels of the participant fall following the start of the seated experimental period (see Appendix VII for details). It is also possible that the use of an eyes-closed resting baseline measure may be inappropriate as a resting baseline measure, and which may have contributed to this observation in both this study and the pilot trial described in Chapter 5. Changes in activation of several brain areas including the visual cortex (Uludag et al., 2004) and ocular-motor system (that includes prefrontal, parietal and occipital cortices - Marx et al., 2004) varies between eyes-closed and eyes-open resting states. As regards the current paradigm, this phenomenon requires further investigation. Even so, the data clearly show that, compared to placebo, concentrations of THb and O2Hb were higher in the DHA-rich FO treatment groups, suggesting modulation of task-related cerebral hemodynamic response following DHA supplementation.

In conclusion, supplementation with DHA-rich FO in non consumers of oily fish appears to modulate task-related regional cerebral blood flow response, as evidenced by the observed differences between cerebral hemodynamic parameters in the active treatment groups compared to placebo. Moreover, the results suggest that modulation may occur in a dose-response manner. To the author's knowledge, this is the first study to examine the parallel effects of fish oil supplementation on cerebral hemodynamics and behavioural outcomes and as such has highlighted that DHA may impact cerebral perfusion of activated areas in healthy young adults without consistent effects on behaviour. Combining imaging techniques in the future could further elucidate the relationship between brain physiological function and behaviour and how dietary intake of n-3 PUFAs affects both of these outcomes, with a view to inform prophylactic treatment of cognitive decline and dementia.

CHAPTER 7. DISCUSSION

7.1 Summary of the objectives of the thesis

The aim of this thesis was to investigate the relationship between dietary n-3 PUFAs and behaviour and brain function, and to evaluate their efficacy for cognitive enhancement in healthy volunteers. Prior to this project's inception, the majority of research evaluating the relationship between n-3 PUFAs and behaviour had focused on mood disorders (e.g. Peet and Horrobin, 2002b, Marangell et al., 2003, Peet, 2003, Su et al., 2003, Fux et al., 2004, Silvers et al., 2005) and on childhood developmental disorders (e.g. Richardson and Montgomery, 2005, Richardson and Puri, 2002, Stevens et al., 2003, Hirayama et al., 2004, Voigt et al., 2001). However, also at the time a number of widely reported 'successes' of fish oil supplements on healthy schoolchildren's performance were gaining a great deal of media attention and the UK government was exploring the possibility of providing n-3 PUFA supplements to all children (Oakeshott, 2006), though no study that the author is aware of had empirically addressed the issue of n-3 PUFAs for cognitive enhancement in normal healthy children. Chapter 2 of this thesis therefore investigated the effects of two doses of DHA on a range of cognitive tasks and mood in healthy schoolchildren.

In addition to this, very limited data also existed concerning the relationship between dietary n-3 PUFAs and cognitive function and mood in healthy adults. Only one study had investigated the effects of fish oil and behavioural outcomes in healthy young adults, with the results revealing that fish oil supplementation may improve performance on tasks requiring attention and some aspects of mood, though the data were rather inadequately analysed making firm conclusions about the efficacy of the treatment difficult to draw (Fontani et al., 2005). To this end, Chapter 3 explored the associations between peripheral PUFAs and cognitive performance and mood in healthy young adults, and Chapter 4 went on to evaluate the effects of dietary supplementation with DHA-rich and EPA-rich FO in a similar sample.

Examination of the literature also revealed that very little attention had been given to investigating the effects of n-3 PUFAs on brain function in humans. In order to

address this gap in the knowledge, Chapter 5 explored the effects of DHA-rich and EPA-rich FO on cerebral hemodynamics, and Chapter 6 extended these findings by evaluating dose-response effects of DHA-rich FO on these parameters and cognitive measures in parallel.

7.2 The relationship between dietary n-3 PUFAs and cognitive function and mood

7.2.1 Children

The results described in Chapter 2 do not favour the use of either 400 mg or 1000 mg DHA for cognitive or mood enhancement in school-aged children. There was no evidence of an effect of either treatment following 8 weeks dietary supplementation on any of the cognitive measures that were employed, and a single treatment effect on the mood questionnaire item 'alert', although further analysis revealed that neither treatment group differed significantly compared to placebo on this measure on Day 56. The failure of the intervention to produce any interpretable effects of DHA supplementation on cognitive and mood outcomes cannot readily be attributed to the sophisticated cognitive testing employed, given that the measures utilised in this study have previously been shown to be sensitive to dietary manipulations in similar samples of healthy children (Haskell et al., 2008, Ingwersen et al., 2007). Instead, it is possible that a number of methodological factors contributed to the null findings of this study, including the length of the treatment intervention, the treatment formulation, being a predominantly DHA supplement, and the nature of the study sample itself, being drawn from a healthy, cognitively intact population. Reference to subsequent intervention studies in healthy children allows more light to be shed on these potential issues. As regards the issue of the length of the intervention, the results of Ryan and Nelson (2008) suggest that 400 mg DHA, even when administered for a longer period of 4 months, is not effective in modulating cognitive function in 4 year old children, suggesting that the nature of the supplement might be more important than treatment duration. Results from other studies carried out in children with ADHD (Hamazaki and Hirayama, 2004, Voigt et al., 2001) that have administered DHA in isolation have also been ineffective at modulating behavioural outcomes, indicating perhaps that effectual n-3 PUFA formulations must include both DHA and EPA, reflecting more naturally occurring

ratios, to produce measurable behavioural effects. On the other hand, the two studies that have administered both DHA and EPA do not provide sufficient evidence to conclusively assert that administration of DHA in isolation is the reason why no effect of treatment was detected in Chapter 2. Osendarp et al. (2007) found no effect of 12 months n-3 PUFA supplementation (88 mg DHA + 22 mg EPA) on measures of general intelligence, learning and attention compared to placebo. Although the quantity of n-3 PUFAs was lowest in this study compared to the others conducted in the area, compared to placebo the peripheral n-3 PUFA status of children receiving the active treatment was significantly greater. In contrast, the study described by Dalton et al. (2009) administered a higher dose of n-3 PUFAs, though less than the study described in Chapter 2 (192 mg DHA + 82 mg EPA, equivalent to 2 portions of medium fat fish per week), in the form of a fish flour spread for 6 months in 7-9 year olds. A beneficial effect of treatment on measures of learning, memory and spelling was reported. However, considering that this study is the only one out of the four studies conducted in this particular area (Osendarp et al., 2007, Ryan and Nelson, 2008, Chapter 2) to show a benefit of n-3 PUFAs over placebo on cognitive measures does not in itself provide adequate evidence to support n-3 PUFA supplementation in children for cognitive enhancement. Taking the results of all the studies together, along with the results reported in Chapter 2, one interpretation of the available data is that n-3 PUFA supplementation does not have any measurable effects on behavioural outcomes in healthy, normally developing children. This conclusion goes against the popularly held, but currently unsupported notion that n-3 PUFAs and fish oils will enhance children's performance. This belief—evidenced by the number of widely publicised 'band-wagon' trials (e.g. "Fish oil study's GCSE successes" 2006; "Pupils test fish brain food pills" 2006) that followed the publication of the 'Oxford-Durham Trial' (Richardson and Montgomery, 2005)—may in part have been bolstered by over-interpretation of the results of the study, and the subsequent promotion of n-3 PUFAs by n-3 PUFA manufacturers (e.g. Equazen) and food companies (e.g. St. Ivel) alike, claiming that their products can enhance children's learning and concentration (ASA, 2007, Oatts, 2006), claims which have had to be subsequently withdrawn following complaints to the Advertising Standards Agency (e.g. ASA, 2006, ASA, 2007). This is not to say that research in this area should not be pursued further. One interesting aspect of the study published by Dalton et al. (2009) is that the children in this study were from low socioeconomic backgrounds

and according to the authors ate virtually no fish, lean or fatty. One possibility could therefore be that n-3 PUFA supplementation may only enhance performance in children not following a healthy balanced diet that includes fish meals. The findings reported by a recent epidemiological study from Sweden would certainly support the hypothesis that a diet incorporating fish meals on a regular basis is associated with better cognitive performance, which they found to be the case in adolescents (18 years) regardless of socioeconomic background (Aberg et al., 2009), suggesting a unique role for dietary fish intake in optimal cognitive function. Overall the results presented in Chapter 2 do not support short-term use of a DHA supplement in school-aged children for cognitive or mood enhancement. Longitudinal intervention trials may be more fruitful in advancing our knowledge regarding the relationship between dietary n-3 PUFAs and cognitive function in children.

7.2.2 Adults (18-35 years)

7.2.2.1 Cognitive function

Two clear patterns of results emerged from the data presented in Chapter 3, in which the relationship between peripheral PUFA status and cognitive function and mood was evaluated. Firstly, the data revealed that serum concentrations of total n-3 PUFAs and EPA were associated with better performance on a cluster of episodic memory tasks (see Table 3.2 for details). The second pattern revealed that the ratio of serum concentrations of AA:EPA was associated with worse performance across several cognitive domains (see Table 3.2 for details). Both of these findings are novel. As regards the former, previous studies that have utilised episodic memory tasks to investigate the relationship between n-3 PUFAs and cognitive function in elderly adults do not report any associations between concentrations of any n-3 PUFAs and performance on these tasks (Dullemeijer et al., 2007, Beydoun et al., 2007, Kalmijn et al., 2004a). However, Dalton et al. (2009) do report significant associations between DHA, total n-3 PUFAs and the ratio of n-6:n-3 PUFAs and performance on a memory task in 7-9 year-olds following a 6-month dietary intervention. The finding that serum concentrations of AA:EPA were inversely associated with performance on a number tasks requiring working memory, episodic memory and attention is, to the author's knowledge, the first report of an association between the ratio of AA:EPA and specific cognitive functions. Previous literature

implicates the balance of these two fatty acids in a range of health and behaviour outcomes including, for example, depression (Adams et al., 1996), reading ability in dyslexics (Cyhlarova et al., 2007) and inflammatory disease (reviewed in Calder, 2006a). This finding suggests that the AA:EPA ratio may potentially be an important consideration as regards cognitive function, though this would need to be confirmed in future studies. Overall the results described in Chapter 3 suggest that peripheral fatty acid status, an index of dietary n-3 PUFA intake, may be linked to specific cognitive functions in healthy adults. However, the results provided by the study described in Chapter 4 do not suggest that increasing intake of n-3 PUFAs has any effect on cognitive function in this population.

The study described in Chapter 4 utilised an identical cognitive task battery as utilised in Chapter 3 to evaluate the effects of 12 weeks' dietary supplementation with either 1 g/d DHA-rich (450 mg DHA + 90 mg EPA) or EPA-rich FO (300 mg EPA + 200 mg DHA) on cognitive performance, in a similar sample of healthy adults. Out of the many cognitive performance outcomes that were assessed, only two main effects of treatment were detected. Participants in the DHA-rich FO treatment group responded faster to stimuli on the Stroop task, and participants in both treatment groups correctly matched fewer names to faces on the Names-to-Faces Recall task (see Section 4.3 for details). From these data it is parsimonious to conclude that supplementation with 1 g/d DHA-rich or EPA-rich FO does not have any interpretable effect on cognitive function in healthy adults. Taking the results of Chapter 3 and 4 together reveals that there is little continuity between the two studies, reflecting the findings of other studies in the field that have shown significant associations between dietary n-3 PUFA intake or n-3 status and cognitive function, yet supplementation studies have failed to produce consistent results. For example, Aberg et al. (2009) reported a positive association between regular fish meals and overall cognitive performance in healthy adolescents (18 years), and in older adults plasma n-3 PUFA concentrations are inversely associated with decline information processing speed (Dullemeijer et al., 2007, Beydoun et al., 2007) and verbal fluency (Kalmijn et al., 2004a). However, only one (Fontani et al., 2005) out of four (Hamazaki et al., 1996, Rogers et al., 2008, Antypa et al., 2009) published studies in healthy adults has shown a benefit of the intervention upon cognitive outcomes. It is possible that the dissimilarity between the correlational and causal evidence provided by the literature in the area may be due to either the methodological variation between intervention studies which has differed in terms of

outcome measures, treatment dose and formulation, and duration of the intervention. On the other hand, it is also possible that the effects of n-3 PUFA supplements on behaviour, should they exist, are very subtle, and that a longer period of intervention is required if these effects are to be observed. A number of longitudinal intervention studies investigating the effects of n-3 PUFAs on cognitive function are currently being conducted in older adults (Dangour et al., 2006, Gillette, 2009, Martek, 2006), the results of which may be able to further elucidate this issue. As regards the current thesis, a limitation of the study described in Chapter 4 is that although there was a significant within group increase in serum concentrations of EPA at Week 12 compared to Baseline in the EPA-rich FO group, at Week 12 there was no evidence of a significant difference between serum concentrations of EPA in this group compared to placebo (see Section 4.3.1), which may be a contributing factor to the null results that were reported. However, given that the overall results are in keeping with other studies in the area, it is more likely that short-term (i.e. 12 weeks) dietary supplementation with n-3 PUFAs does not impact upon cognitive function in healthy adults.

Results from the cognitive performance outcomes assessed in the study described in Chapter 6 revealed that participants in both treatment groups (1 g and 2 g/d DHA-rich FO) were faster than placebo on the Choice Reaction Time task and those in the 2 g group were faster on the RVIP task following 12 weeks daily supplementation. Taken as a single investigation, these results again do not provide strong evidence to support the use of n-3 PUFA supplements for cognitive enhancement in healthy adults. However, taking the results of Chapters 4 and 6 together, a similarity in the results of both studies is that, compared to placebo, participants administered the DHA-rich FO (the same treatment formulation was administered in both studies), responded faster to stimuli on attention tasks. What is more, if these results are considered along with the available data from published studies that have also assessed the effects of n-3 PUFAs on cognitive outcomes, a pattern in the results can be identified. To illustrate, data from fish oil supplementation studies in other adult samples suggests that impulsivity (Rogers et al., 2008) and tasks requiring response inhibition and sustained attention (Fontani et al., 2005) may be modulated by n-3 PUFAs. It is also interesting that children diagnosed with ADHD, a developmental disorder characterised by impulsivity and inattention (Pary et al., 2002), have lower tissue concentrations of n-3 PUFAs (DHA and EPA) (Burgess et al., 2000, Stevens et al., 1995), and there is some evidence

to suggest that symptoms of ADHD are ameliorated following dietary supplementation with n-3 PUFAs (Stevens et al., 2003, Richardson and Montgomery, 2005, Belanger et al., 2009, Richardson and Puri, 2002). What all the available research may collectively be beginning to indicate is that dietary n-3 PUFAs, and from the studies described in this thesis DHA in particular, may modulate mechanisms that impact upon performance on attention tasks in healthy adults and break down in the case of neurodevelopmental disorders. This being said, it is noteworthy that serum concentrations of DHA were not associated with attention task outcomes in the study described in Chapter 3. Even so, the evidence provided by Chapters 4 and 6 does merit further investigation. To better understand the role of n-3 PUFAs in cognitive function, a future study could compare the effects of DHA-rich FO supplementation on a small number of attention/impulsivity tasks (e.g. CRT, Stroop, RVIP, digit vigilance, go/no go etc.). In addition, given that animal evidence indicates the possibility of a 'threshold' of intake/tissue incorporation, below which deficits are observed and can be subsequently modulated via supplementation (Jensen et al., 1996), an informative, albeit costly feature of such a study would recruit participants on the basis of low peripheral fatty acid concentrations.

It is beyond the scope of the evidence provided by this thesis to conclusively assert specific mechanisms to which these observed effects on behavioural outcomes could be ascribed, especially given the many known actions of DHA in the brain (see Chapter 1.4). One plausible candidate may be modulation of neurotransmission pathways; studies conducted in animals have demonstrated that alterations in various neurotransmitter pathways including cholinergic, serotonergic and dopaminergic transmission occurs when n-3 PUFAs are absent from the diet (Zimmer et al., 2000b, Zimmer et al., 2000a) and subsequently replenished (Chalon et al., 2001, Delion et al., 1996, Kodas et al., 2004), and is reflected in task performance. For example, cholinergic transmission is associated with sustained attention task performance in rats (Himmelheber et al., 2000), which has been shown to be modulated by dietary n-3 PUFAs (Tsukada et al., 1997). Alterations in serotonergic transmission could be another candidate given that fish oil supplementation in n-3 PUFA deficient rats results in increased levels of serotonin in the frontal cortex (Chalon et al., 1998), and serotonin has been shown to be directly involved in sustained attention tasks in humans (Wingen et al., 2008). Further, chronic n-3 PUFA deficiency in rodents has been shown to lead to reduced

levels of dopamine and binding at D2 receptors in the brain and was accompanied by attentional dysfunctions in behaviour, not dissimilar to symptoms displayed by children with ADHD (Zimmer et al., 2002). Of course, only other types of imaging techniques (e.g. PET, fMRI) would be able to identify modulation of specific pathways by dietary intake of fish oil, and presents an interesting avenue for future research in the area.

Overall, the results presented in this thesis suggest that the relationship between dietary n-3 PUFAs and cognitive function in healthy adults is complex, and has yet to be fully explored. The results from the intervention studies potentially indicate a role for DHA in attention task performance, however cautious interpretation of these sparse cognitive performance results should be exercised considering that both intervention studies analysed the effects of the interventions on a large number of performance outcomes.

7.2.2.2 Mood

The results of the intervention study described in Chapter 4 did not reveal a significant effect of either EPA-rich or DHA-rich FO on any of the assessed mood outcome measures (Bond-Lader, DASS). Previous intervention studies that have evaluated the effects of n-3 PUFAs on mood in healthy adults have revealed inconsistent results. Both Fontani et al. (2005) and Antypa et al. (2009) used the Profile of Mood States to assess mood. While the former reported within-group differences regarding increased vigour along with reduced anger, fatigue, confusion, anxiety and depression, the latter only reported an effect of treatment on subjective ratings of fatigue, compared to placebo. In addition, Rogers et al. (2008) found no effect of treatment on any of the subscales of the DASS, and no significant associations between baseline plasma PUFA concentrations and these outcomes at baseline. Depression in particular has received a great deal of attention in the literature, and whilst fish consumption (Hibbeln, 1998, Tanskanen et al., 2001, Silvers and Scott, 2002) and peripheral n-3 PUFA concentrations (Peet et al., 1998b, Edwards et al., 1998, Adams et al., 1996) are associated with measures of depression, the evidence provided by intervention studies is less than conclusive (reviewed in Appleton et al., 2008, Freeman et al., 2006b). This pattern of findings echoes the observations presented in the previous section in that

epidemiological/cross-sectional and intervention study data do not yield consistent results, and also that there is little consistency within the results provided by intervention studies. A further example of this theme is provided by the finding that the ratio of AA:EPA was positively associated with the Anxiety scale of the DASS in Chapter 3, but this was not modulated by either intervention in Chapter 4, despite the fact that the serum ratio of AA:EPA was significantly higher in the placebo treatment group compared to both the active treatment groups (see Table 3.2 and Section 4.3.1 for details). Again, reasons for this may be down to methodology or simply that the effects of n-3 PUFAs are not manifested in observable behavioural changes.

Having said this, an interesting finding across Chapters 3 and 4 is the relationship with and effect of EPA-rich FO on subjective ratings of during thirty minutes of cognitively demanding tasks (Cognitive Demand Battery - CDB). Chapter 3 demonstrated that the ratio of serum concentrations of AA:EPA was positively associated with mental fatigue on the CDB. Similarly, in Chapter 4 participants assigned to the EPA-rich FO treatment reported less average mental fatigue during the CDB than placebo following 12 weeks daily supplementation, a finding that was not observed in the DHA-rich FO group. Analysis of the serum fatty acids levels of participants in Chapter 4 interestingly revealed that serum levels of EPA at Week 12 did not differ between those taking the EPA-rich FO and placebo. However, reference to the serum data regarding the AA:EPA ratio however revealed that this measure was significantly lower in the EPA-rich FO condition, compared to placebo, although this difference is more likely due to the observed increase in this ratio at Week 12 compared to Baseline in the placebo group (Table 4.2). Taken together, the results of these two studies suggest that in healthy young adults, the endogenous balance of AA and EPA may play an important role in subjective mental fatigue. In addition, dietary supplementation with 1 g/d EPA-rich FO (EPA 3:2 DHA) may be effective at reducing subjective mental fatigue under cognitively demanding conditions. Further, this finding is readily translated into dietary advice and is roughly equivalent to consuming one portion of oily fish per week; herring and some species of salmon in particular have a similar EPA:DHA ratio to the experimental treatment. Given that the current UK advisory already recommends consumption of one portion of oily fish a week (SACN/COT, 2004), it might be predicted based on the findings presented in Chapter 4 that individuals following these guidelines would suffer from less subjective mental fatigue, particularly when

cognitive resources are in high demand. In light of the findings presented by Fontani et al. (2005) and Antypa et al. (2009) who also reported reduced mental fatigue on the POMS following fish oil supplementation, it might also be predicted that regular intake of oily fish may reduce mental fatigue as a general mood state, though this would need to be empirically confirmed.

Although these studies do not provide adequate evidence to irrefutably identify an underlying mechanism, given the serum fatty acid data presented in Chapters 3 and 4, one hypothesis is that the reduced subjective mental fatigue reported by the participants administered the EPA-rich FO could be attributed to modulation of inflammatory lipid mediators by this intervention. There is evidence to suggest an association between fatigue and elevated cytokines, such as in the case of chronic fatigue syndrome (reviewed in Patarca, 2001), as well as evidence demonstrating that plasma n-3 PUFAs are associated with lower levels of pro-inflammatory cytokines, in part due to the inhibitory effect EPA-derived prostaglandins have on the production of certain cytokines (Bagga et al., 2003, Miles et al., 2002). It is also known that increased intake of dietary EPA leads to increased incorporation of these molecules into membrane phospholipids, in a dose response manner and at the expense of membrane incorporation of AA, resulting in a shift away from a pro-inflammatory phenotype to an anti-inflammatory one (Calder, 2007). Based on this evidence, one possibility is that administration of EPA-rich FO attenuated a further elevation in the ratio of AA:EPA (given the serum fatty acid data), which in turn may have modulated participant's immunological response to laboratory-induced mental fatigue, resulting in the significant effect of the EPA-rich FO on the subjective visual analogues scale. Considering the ratio of AA:EPA was also lower than placebo in participants administered the DHA-rich FO, it is also possible that the administration of a higher amount of EPA (300 mg as opposed to 200 mg) in the EPA-rich FO treatment group may be the basis for this effect. Naturally these hypotheses would have to be verified in future investigations, and could be achieved by including a variety of immunoassays.

In summary, the evidence from this thesis does not suggest that dietary supplementation with n-3 PUFAs modulates subjective ratings of alertness, calmness, contentedness or depression, stress and anxiety in healthy young adults. On the other hand, the data provided by Chapters 3 and 4 suggest that subjective ratings of mental fatigue during cognitively demanding tasks may be sensitive to

dietary n-3 PUFAs; supplementation with EPA-rich FO appears to reduce subjective mental fatigue, and may be linked to an endogenous balance of AA and EPA, though this requires further investigation.

7.3 Cerebral hemodynamics

The studies presented in Chapters 5 and 6 of this thesis used Near Infrared Spectroscopy (NIRS) to assess the effects of n-3 PUFAs on cerebral hemodynamics during cognitive task performance following 12 weeks dietary supplementation in healthy young adults. Overall the results revealed that cerebral blood flow (CBF) is increased in the prefrontal cortex during tasks that activate this brain region in participants administered DHA-rich FO compared to placebo, as evidenced by the increased task-related concentration changes in oxygenated haemoglobin (O2Hb) and total haemoglobin (THb). These findings provide the first evidence of modulation of cerebral hemodynamics by n-3 PUFAs in human participants.

The results of the pilot study described in Chapter 5 revealed that cerebral hemodynamic response to tasks was different from placebo only in the DHA-rich FO treatment group, and not in the EPA-rich FO treatment group. More specifically, concentration changes in O2Hb and THb were higher in the DHA-rich FO group compared to placebo. Given these findings were not also observed in the EPA-rich FO treatment group, suggests that cerebral hemodynamic response to tasks is modulated by DHA and not EPA. This may be due to the minimal amount of EPA contained in the brain (McNamara and Carlson, 2006), despite the fact that EPA is known to have beneficial effects elsewhere in the cardiovascular system (e.g. endothelial cell function - Hashimoto et al., 1999, Matsumoto et al., 2009). Of course it is possible that at very high doses of EPA, retro-conversion to DHA could occur and in this way indirectly beneficially impact upon these parameters, although this would need to be investigated further. Overall this small pilot trial established the possibility of a link between n-3 PUFAs and physiological brain function in humans, which has, to date, received little attention.

The findings revealed in Chapter 6 confirmed the results of the pilot study in that compared to placebo, participants' cerebral hemodynamic response to tasks was modulated following dietary supplementation with DHA-rich FO with an increase in

rCBF being observed. In addition, this study identified that the task-related concentration changes in O2HB and THb occurred in a dose-response pattern. Taking the results from both studies together, the evidence suggests that in both consumers (Chapter 5) and non consumers (Chapter 6) of oily fish, administration of DHA-rich FO is associated with increases in concentrations of both THb and O2Hb in response to cognitive tasks, compared to placebo. Both O2Hb and THb (the sum of O2Hb and HHb) have been proposed as indices for rCBF; Hoshi et al. (2001) revealed that in rat brain perfusion models O2Hb was the most sensitive indicator of rCBF however Steinbrink et al. (2005) demonstrated that concentrations of THb always correspond to cerebral blood volume, which closely follows the same pattern as CBF. Given that DHA-rich FO modulated both these parameters in the same direction it could be concluded from these data that DHA-rich FO modulates the rCBF response to local brain activity.

In humans, the relationship between n-3 PUFAs and physiological brain function has only been addressed, to the author's knowledge, by two studies, the results of which also support a link between n-3 PUFAs and brain function. For example, Hirashima et al. (2004) reported evidence of a dose-response effect of n-3 PUFA supplementation on whole-brain T2 relaxation times (indicative of increased activation following treatment) in patients with bipolar disorder, although any effects of the treatment on this measure in normal control subjects were not assessed. In addition, in an attempt to identify which areas of the brain might be more susceptible to n-3 PUFA deficiency, Sublette et al. (2009) established that peripheral concentrations of DHA (and AA) but not EPA were associated with regional glucose metabolism (as measured by PET) in depressed patients. Both of these studies suggest that dietary n-3 PUFAs may be associated with aspects of brain function, though the nature of this relationship has yet to be fully explored. In relation to the studies described in Chapters 5 and 6, it is a study conducted in rhesus monkeys that is the most relevant. In this study, aged monkeys (mean age 18 years) were administered either 150 mg/kg/d DHA for 4 weeks or a soybean milk placebo (Tsukada et al., 2000a). Following the intervention it was discovered that animals administered the active treatment had an increased rCBF response to tactile stimulation (as measured by PET), compared to placebo. The magnitude of this response was 133% of the rest condition, compared to 116% in the placebo group. What is more, a previous study by the same group established that this rCBF response was reduced in aged animals compared to young ones (116% of rest

condition compared to 144%, respectively), suggesting that the DHA supplementation was able to restore age-related decreases in rCBF response (Tsukada et al., 2000b). Given these findings in aged monkeys, along with the results presented in Chapters 5 and 6, an exciting avenue of research to pursue could evaluate the effects of DHA-rich FO on cerebral hemodynamics in elderly human participants. Cerebrovascular insufficiency is associated with a number of neurodegenerative conditions including vascular dementia and Alzheimer's disease, but is also a feature of normal ageing (Farkas and Luiten, 2001). At the epidemiological level, increased consumption of fish and/or higher plasma levels of n-3 PUFAs is associated with reduced risk for cognitive decline (Kalmijn et al., 1997a, Kalmijn et al., 2004b, Dullemeijer et al., 2007, Heude et al., 2003), dementia (Kalmijn et al., 1997b, Cherubini et al., 2007) and Alzheimer's disease (Conquer et al., 2000, Morris et al., 2003), and dietary supplements containing n-3 PUFAs are currently being trialled in several longitudinal studies as a treatment for preventing cognitive decline and dementia in the elderly (Dangour et al., 2006, Gillette, 2009, Martek, 2006, Quinn, 2007). To date, results from previous intervention studies have not been successful (Terano et al., 1998, van de Rest et al., 2008, Freund-Levi et al., 2008), but it is hoped that data from the these on-going large-scale longitudinal trials may reveal more promising results, given the methodological variation between the previous studies that has rendered interpretation of findings in the field as a whole somewhat challenging (Fotuhi et al., 2009). It is interesting that research conducted in 2VO (a model of cerebral hypoperfusion, de Wilde et al., 2002) and hypertensive rats (de Wilde et al., 2003) has demonstrated that while n-3 PUFA administration is effective at improving vascular parameters, these physiological benefits are not reflected in behavioural outcomes, where task performance is similar between intervention and control animals. A similar finding was observed in Chapter 6, where there is evidence of an effect of DHA-rich FO on brain function (rCBF response to tasks), without consistent effects on behaviour. It appears that the observed effects of n-3 PUFA supplementation on cerebral hemodynamics are too subtle to affect behaviour in healthy young adults. However, given the evidence from epidemiological studies linking fish consumption/n-3 PUFA status with reduced cognitive decline and incidence of dementia in the elderly suggests perhaps that the cerebral hemodynamic effects of n-3 PUFAs reported in this thesis, if present over the course of a lifetime, may indeed impact behavioural outcomes, and that long-term intake of n-3 PUFAs are required for these effects to

be observed. Of course, this suggestion would require further exploration, and the investigation of the cerebral hemodynamic effects of n-3 PUFAs in an elderly adult sample could comprise a valuable avenue of future research.

7.4 Dietary advice on n-3 PUFA intake

At present, a dietary reference value (DRV) for DHA and EPA has not been established. Efforts to do so have been faced with a number of challenges concerning the nature of n-3 PUFAs themselves, along with the type and quality of research that has been conducted to inform such decisions (Harris et al., 2009). The focus of the advice given by all international recommendations concerns the prevention of cardiovascular disease, with no mention of required intake in relation to cognitive function past infancy (Harris, 2007). The current advisory in the UK is that children and adults should aim to eat 2 portions of fish a week, one of which should be oily, to meet a minimum intake of 450 mg/d (SACN/COT, 2004). Based on this, the intervention studies described in Chapters 4, 5 and 6 administered doses of fish oil which are roughly equivalent to this dietary recommendation so that any results could be translated into meaningful dietary advice. The results presented in Chapter 4 suggest that, in low consumers of oily fish (mean intake 1.46 portions/month), the addition of another portion of oily fish per week, or 1 g/d fish oil supplement, is not likely to confer an appreciable benefit on cognitive performance or mood, although the results presented in Chapters 5 and 6 suggest that this dose may have an effect on physiological brain function (cerebral hemodynamic response to tasks). This latter finding particularly suggests that even in the absence of any observed behavioural effects, increased intake of dietary n-3 PUFAs does appear to impact brain function. Given that epidemiological studies indicate that long-term consumption of fish (and presumably n-3 PUFAs), is associated with reduced risk for cerebral insufficiency-related conditions including cognitive decline and dementia (e.g. Morris et al., 2005, Dullemeijer et al., 2007, Barberger-Gateau et al., 2007), it is possible that the increased rCBF response to tasks observed in the current thesis may be a beneficial effect of n-3 PUFAs. If so, the dose-response pattern observed in Chapter 6 regarding the cerebral hemodynamic effects of DHA-rich FO suggests that whilst current recommendations are likely to meet requirements for optimal cognitive function in healthy individuals, dietary intake of n-3 PUFAs for optimal cerebrovascular function has yet to be established.

On a related note, the wider issue regarding the sustainability of the increased fish intake promoted by dietary guidelines needs to be addressed. The marine environment is already under immense pressure, and health policies that recommend that we eat more fish directly collide with the environmental issue of depleted fish stocks due to overfishing the world over (Brunner et al., 2009). It is hoped that advances in plant biotechnology will be able to offer a solution to these issues. Production of DHA from various algal species including *Schizochytrium sp* has proved to be very successful and DHA produced in this way is used in numerous supplements and infant formulas (Ward and Singh, 2005). Production of EPA from algae is presently less fruitful, and consequently vegetarian sources of EPA contain limited amounts of this n-3 PUFA, however research is still on-going to increase algal production of EPA (reviewed in Ward and Singh, 2005). These forms of algal DHA and EPA are safe and well-tolerated (Innis and Hansen, 1996, Arterburn et al., 2000), and in the future could potentially be used to help meet human dietary requirements for long-chain n-3 PUFAs.

In relation to this, a number of 'smart' or 'functional' foods containing added omega-3 are currently being sold in the UK. Products include milk (e.g. St Ivel Fresh Milk with Omega-3), yogurt (e.g. Muller Vitality Pro-biotic drinks), bread (e.g. Kingsmill 50/50 with Omega-3) and spreads (e.g. Flora Omega-3 Plus). UK consumer group Which? warns consumers to be wary when purchasing such projects, as the quantity of n-3 PUFAs contained within is often minimal, and large quantities of these foods would need to be consumed on a daily basis to meet the current guidelines (Which, 2007). Dietary decisions made by consumers to increase their intake of n-3 PUFAs via these novel sources are unfortunately not being facilitated by European Commission legislation. Recent laws regarding food and beverage label claims could be potentially misleading given that a product can now state that it is a 'source of' or 'high in' n-3 PUFAs regardless of whether the n-3 PUFAs are derived from plant (ALA) or marine (DHA or EPA) sources, although this distinction would have to be made elsewhere on the label [Commission Regulation (EU) No 116/2010]. Given that the conversion rate of ALA to DHA and EPA is low (Burdge et al., 2003), and that DHA and EPA are more biologically relevant to fundamental cellular processes, disease prevention and overall health (see Sections 1.4 and 1.5.2), it would seem that product label claims require further qualification. Further to this, the European Food Safety Authority recently published a Scientific Opinion document proposing a labelling reference intake value of 250 mg/d for DHA+EPA

(ESFA, 2009), that directly conflicts with the advice of the UK government's (SACN/COT, 2004) and other international scientific committee guidelines (e.g. ISSFAL, 2004). A public consultation on this document is currently on-going, and it is hoped that these issues will be adequately addressed. Overall, it is evident that more work is required in order to produce more detailed, accurate and helpful advice on recommended dietary intake of n-3 PUFAs.

7.5 Limitations

Although the studies that make up this thesis present novel data regarding the relationship between dietary n-3 PUFAs and behaviour in healthy individuals, it is also essential to consider some potential methodological limitations, some of which have only been briefly mentioned in previous chapters.

One potential issue arises from the large number of outcome measures that were employed in Chapters 2, 3 and 4, and the inevitable increased risk of detecting significant effects by chance. For the study described in Chapter 2, a comprehensive battery of cognitive tasks was employed on the premise that no research had previously been conducted investigating the effects of n-3 PUFAs on cognitive function and mood in healthy children, from which educated decisions about particular tasks to utilise could be made. Both the CDR and Internet batteries have been shown to be sensitive to dietary manipulations in children (Haskell et al., 2008, Ingwersen et al., 2007), so these were employed on their strength of being able to assess performance easily and effectively across multiple cognitive domains. This same approach was adopted in Chapters 3 and 4 in the exploration of the relationship between n-3 PUFAs and cognitive function and mood in healthy adults. In recognition of this potential limitation, interpretation and discussion of the results throughout this thesis has focused on those results that fall into patterns, rather than discussing every result at length. In relation to this, the choice of planned comparisons for the analysis of the effects of the treatment interventions on cognitive function in Chapters 2, 4, and 6 should also be discussed. The strictly planned comparisons assessed a limited number of questions of true relevance i.e. the effect of the active treatments on cognitive function/mood i.e. the effect of each treatment versus placebo at the post-dose assessment, and is an approach that is advocated by Keppel (1991). However, the fact that the combination of numerous

outcome measures analysed using a powerful (i.e. least conservative) statistical approach resulted in the detection of only a limited number of significant results suggests that a Type II error has not been committed. Taken from this point of view, the use of multiple outcome measures can instead be seen as a strength of the studies described in this thesis, from which it could be concluded that the behavioural effects of n-3 PUFA supplements on healthy, cognitively intact children and adults, are minimal.

On a related note, the issue of correcting for multiple outcome measures should also be addressed. For example, in Chapter 4, 30 separate dependent variables were analysed. One approach to handling the potential increased chance of a Type I error could be to adjust the required alpha level i.e. 0.05/30 = 0.0017. If this method had been adopted throughout this thesis, the only significant main effect of treatment that would have been detected is for reaction time on the RVIP task utilised in Chapter 6. Although the findings that were significant at the 0.05 alpha level have been discussed in context of the extant literature throughout this thesis, the overall conclusions that have been drawn are the same as those that would be concluded using a more conservative alpha level i.e. that fish oil supplementation in healthy individuals has no observable effect on cognitive performance.

As mentioned above, the discussion of the significant behavioural findings has focused on describing patterns in the data presented in this thesis, and on drawing parallels between these patterns and the extant literature. A discussion of the potential mechanisms has accompanied these sections. However, it must be openly noted that it is beyond the scope of this thesis to conclusively assert that the suggested modes of action do indeed underpin the significant results that have been described, and have been made with reference to the extant literature. One mechanism that was revealed in the studies described in Chapters 5 and 6 is modulation of cerebral hemodynamic response to tasks by DHA-rich FO. However, this effect was not consistently accompanied by behavioural modification in Chapter 6, highlighting the fact that although DHA may be impacting brain function, this effect is not manifested in behavioural outcomes in this population. Overall, more research into the physiological effects of dietary n-3 PUFAs, and how these are linked to behaviour is required. It is hoped that future research will combine techniques (e.g. imaging and behavioural measures) to further elucidate this issue.

Another methodological consideration concerns the formulation of the fish oil supplements administered in Chapter 4. The aim of this study was to evaluate the effects of DHA-rich and EPA-rich fish oil on cognitive function and mood in parallel, under the same conditions, which has previously never been carried out. It could be argued that a more appropriate comparison would be between formulations with opposite ratios i.e. 5:1 DHA:EPA and 1:5 DHA:EPA, given that the 3:2 EPA:DHA ratio is closer to 1 than the DHA-rich FO. However, the ratios of 5:1 (DHA:EPA, DHA-rich FO) and 3:2 (EPA:DHA, EPA-rich FO) were selected on the basis that they reflect ratios that occur naturally in various oily fish, from which the findings of the study could be readily translated into meaningful dietary advice. The results of Chapter 4 do not favour the use of either formulation for cognitive and mood enhancement. However, the results presented in Chapters 5 and 6 suggest that the DHA-rich FO formulation may be impact brain function as regards cerebral hemodynamic response to tasks.

In relation to this, another limitation of this research concerns the effectiveness of the EPA-rich FO in increasing mean serum levels of EPA in this treatment group significantly above that of the placebo group, although there was evidence of a trend in this direction (see Section 4.3.1). The 1 g/d dose administered to participants in the intervention study described in Chapter 4 was chosen on the basis that the results could be translated into meaningful dietary advice. Therefore, in this sample, 300 mg/d EPA was not a sufficient dose to significantly increase peripheral concentrations. From these results it can be concluded that that the addition of an EPA-rich FO supplement (roughly equivalent to 1 portion of oily fish/week) to individuals consuming some oily fish meals in a month does not confer any benefit on cognitive function. Given the serum fatty acid data, it could also be argued that the effects of EPA supplementation on cognitive function and mood in healthy adults have not been adequately addressed, and requires further investigation. However, given the inconsistent behavioural results reported in studies that have administered EPA to healthy adults at higher doses than in the present thesis (e.g. Antypa et al., 2009, Fontani et al., 2005, Rogers et al., 2008), does not suggest that this avenue of research will be met with more informative findings.

Another limitation relates to the limitations of the NIRS imaging technology itself that was utilised in Chapters 5 and 6. The results presented in these chapters suggest a

consistent effect of DHA-rich FO on cerebral hemodynamic response to tasks, and are among the first studies to utilise NIRS in nutritional intervention studies. However, NIRS only provides data on the relative concentration changes in each of the separate chromophores (O2Hb, HHb, THb) in response to tasks in the region of interest (the prefrontal cortex in the present thesis), and does not provide any information regarding the effects of the treatment on general cerebral blood flow. So although the results presented in Chapters 5 and 6 are promising, the paradigm could be improved in future interventions. Incorporating measures such as Trans-cranial Doppler, for example, that would allow the cerebral blood flow effects of the treatment to be quantified.

A final potential limitation concerns the cognitive tasks that were employed. One possibility is that the tasks were not specific enough to detect any effects of treatment. For example, a significant association between the serum AA:EPA ratio and subjective mental fatigue during cognitively demanding tasks was detected in Chapter 3, and in Chapter 4, ratings of metal fatigue were significantly lower in participants following supplementation with EPA-rich FO. Given these findings, it may be that the effects of n-3 PUFAs on cognitive performance in healthy children and adults, if they indeed exist, may be better observed using much more difficult tasks (e.g. increasing cognitive load using duel task paradigms etc.), than were employed in the current thesis. Inducing mental fatigue at the start of the assessment could comprise a second approach to this issue.

7.6 Future research

As well as addressing some of the limitations of this thesis presented above, there are a number of novel avenues that could be pursued based on the findings revealed by the studies described in this thesis. The first of these considerations concerns the issue of dietary n-3 PUFAs and cognitive function in vegetarians and vegans. Intake of total n-3 PUFAs in vegetarians is approximately 30% lower than in fish-eaters (Welch et al., 2008) and peripheral levels of n-3 PUFAs have been consistently found to be lower in vegetarians and vegans than in omnivores (reviewed in Sanders, 2009). However, children raised on a vegetarian diet are known to develop normally (Sanders and Reddy, 1994), and there is no evidence to suggest that vegetarians are more likely to develop dementia later in life (Giem et

al., 1993). This may be in part due to an adaptive mechanism in which conversion from ALA to DHA is increased (Welch et al., 2008). However, the findings presented in Chapter 6 suggest that vegetarians or indeed non-fish meat eaters comprise a particular population which may benefit from added n-3 PUFAs. Vegetarian supplements containing appreciable amounts of EPA are not currently available, so this would present an issue for future supplementation studies.

The results presented in Chapters 3 and 4 reveal interesting and novel data regarding the relationship between n-3 PUFAs and subjective mental fatigue during cognitively demanding tasks. Although these findings require further exploration, the results suggest that subjective mental fatigue may be associated with the balance of endogenous AA and EPA. This balance can be manipulated via supplementation with n-3 PUFAs, though only supplementation with EPA-rich FO had an effect on mental fatigue, compared to placebo. Regulation of the immune response via dietary PUFAs has been studied and reviewed in detail (e.g. De Caterina and Basta, 2001, Calder, 2006a), as has the relationship between psychological stress and the immune response (e.g. Segerstrom and Miller, 2004), but very little attention has been given to exploring the association between simultaneous modulation of inflammatory parameters and behavioural outcomes. Assessing the effects of dietary fatty acids on psychological stress would be one such opportunity for further research. Administration of the parent fatty acids ALA and LA in a ratio of 4:1 to rats prior to exposure to an acute stressor prevented against elevated cortisol and cholesterol levels (Yehuda et al., 2000). Although the results presented in Chapter 4 did not reveal any evidence of modulation of subjective stress by either DHA-rich or EPA-rich FO, whether supplementation with n-3 PUFAs attenuates physiological and psychological stress reactivity in humans would be worthy of investigation. Laboratory-induced stress and the inclusion of various immunoassays (e.g. cytokines, cortisol etc.) would be key features of this work.

In addition, future research is also required to further develop the preliminary findings presented in this thesis regarding the modulation of cerebral hemodynamic response to tasks by the DHA-rich FO. The question of whether administration of DHA-rich FO could similarly modulate task-related rCBF in elderly volunteers, and possible associations with cognitive performance, remains to be demonstrated. This research would be particularly pertinent given that a number of on-going longitudinal

prospective intervention trials are currently evaluating the efficacy of n-3 PUFAs on cognitive decline and dementia.

Finally, the emerging area of nutritional genomics, or nutrigenomics, may be particularly relevant to future investigations of the effects of dietary n-3 PUFAs. Nutrigenomics is concerned with understanding the influences of dietary factors on the genome (the sum total of all the genetic information in an organism), and exploiting the knowledge of these interactions to improve strategies in the prevention and treatment of chronic disease, or indeed to maximise one's genetic potential (Debusk et al., 2005). Dietary PUFAs are known to influence gene expression (see Section 1.4.3), the impact of which on health and behavioural outcomes in humans has yet to be fully understood. For example, there are a number of known genetic variations of the Interleukin-1 (IL-1, a cytokine) gene cluster, some of which are associated with increased severity of inflammatory diseases including Alzheimer's disease (Mrak and Griffin, 2001) and rheumatoid arthritis (Lee et al., 2004). Levels of IL-1 are suppressed following fish oil supplementation (Endres et al., 1989), and so a greater understanding of the effects of these particular dietary compounds in individuals with a pro-inflammatory genetic predisposition could greatly aid the development of effective intervention strategies. In a similar vein, the possibility that dietary interventions do not have the same effects on all individuals must also be considered. For instance, female carriers of the G/A allele on the APOE1 gene respond differently in terms of HDL cholesterol levels following n-3 PUFA supplementation (Ordovas et al., 2002). Overall it seems plausible that other, currently unknown, genetic polymorphisms or genotypes could interact with dietary intake of n-3 PUFAs, with varied effects on behavioural outcomes. It is hoped that the potential for future enquiry in this area to improve quality of life at both an individual and epidemiological level is realised.

7.7 General conclusions

The commercial market for n-3 PUFA supplements includes products such as 'Eye q mind' and 'Brain Boosters'. The claims that these product labels can contain is governed by legislation (see Section 7.4), but the names in themselves are unambiguous. The primary aim of this thesis was therefore to empirically investigate the relationship between dietary n-3 PUFAs and cognitive function and mood in

healthy individuals, and specifically to evaluate their efficacy as a cognitive enhancer. The results from the intervention study described in Chapter 2 do not suggest a beneficial effect of either 400 mg or 1000 mg DHA on cognitive performance or mood in healthy children. This finding is important as it goes against the unfounded, but commonly held belief regarding the cognitive enhancing properties of n-3 PUFAs in children. Limitations of this study include the nature of the formulation and the duration of the intervention, however given that only one study (Dalton et al., 2009) has reported a benefit of n-3 PUFAs on cognitive function in children, the available evidence does not support the use of n-3 PUFA supplements for cognitive or mood enhancement in healthy, normally developing schoolchildren.

As regards the relationship between n-3 PUFAs and cognitive function in healthy adults, the findings presented in Chapter 3 indicating a positive association between serum concentrations of total n-3 PUFAs and EPA and performance on a cluster of episodic memory tasks. However, EPA-rich FO does not appear to have any cognitive enhancing properties. A limitation of the study described in Chapter 4, in this respect however, is that the dose of EPA administered was not effective at raising serum EPA levels above that of those administered the placebo treatment; consequently the issue of EPA supplementation and cognitive function may require further attention. Overall, the findings of the studies presented in this thesis suggest that the cognitive enhancing effects of n-3 PUFAs in healthy adults are also minimal. The pattern of modulation of attentional task performance following supplementation with DHA-rich FO reported in Chapters 4 and 6 should not be ignored, however further investigation this finding is recommended.

In both healthy children and adults, supplementation with n-3 PUFAs does not have any measurable effect on subjective mood, however dietary n-3 PUFAs may be associated with subjective mental fatigue under cognitively demanding conditions. Although n-3 PUFAs have previously been shown to improve mental fatigue as a general mood state (Antypa et al., 2009, Fontani et al., 2005), this is the first demonstration of reduced mental fatigue induced under laboratory conditions. One possibility is that, given the current evidence regarding the beneficial modulation of various immune responses via increased intake of n-3 PUFAs (Calder, 2006b), an evaluation of the parallel behavioural and immunological effects of n-3 PUFAs could comprise an exciting avenue for future research.

A second aim of this thesis was to assess the effects of n-3 PUFAs on cerebral hemodynamics, given the lack of data describing the effects of n-3 PUFAs on brain function in humans. The results presented in Chapters 5 and 6 revealed that DHA-rich FO modulates cerebral hemodynamic response to tasks in both consumers and non consumers of oily fish. In addition, this pattern of modulation occurs in a dose response manner. Also, it appears that whilst DHA-rich FO has an impact on cerebral hemodynamics, this is not manifested in behaviour modification. These findings are the first demonstration of modulation of cerebral hemodynamics by n-3 PUFAs, and future research in this area could further elucidate the nature of the inverse relationship between intake of n-3 PUFAs and incidence of cerebrovascular disease and cognitive decline (e.g. Farkas and Luiten, 2001, Kalmijn, 2000).

The relationship between dietary n-3 PUFAs and behaviour is complex. Animal and epidemiological and cross-sectional studies in humans suggest that there are behavioural consequences of low intake of n-3 PUFAs. It has been argued that a typical Western diet does not incorporate adequate amounts of n-3 PUFAs (Simopoulos, 2002); this is certainly true of the UK, where the last National Diet and Nutrition Survey revealed that 74% of the population does not eat any oily fish, a principal source of DHA and EPA (Henderson et al., 2002). In healthy children and adults not consuming appreciable amounts of oily fish, short-term supplementation (8-12 weeks) with n-3 PUFAs has limited effects on behaviour. However, in the absence of concurrent effects on behavioural measures, the findings presented in this thesis suggest that supplementation with dietary n-3 PUFAs can influence physiological fatty acid status and cerebral hemodynamics in healthy adults, the full impact of which on other health and behavioural parameters has yet to be fully addressed, and comprises several exciting possibilities for future research.

APPENDIX I: Depression, Anxiety and Stress Scales

DASS-42

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 (e.g., 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 (e.g., 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 (egg, 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 (e.g., 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 (egg, 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 (e.g., 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 II: Health and diet questionnaire utilised in Chapter 3

Age _____ **Glasses/Contacts Y/N _____**

Sex _____ **Handedness L/R _____**

Weight _____ lbs

Height _____ ft _____ ins

1) Are you a vegetarian or vegan? No • Vegetarian • Vegan •

2) How many portions of fruit and vegetables do you eat on a typical day?
_____

(Juice only counts as 1 portion regardless is you drink multiple glasses)

3) How many portions of oily fish do you eat in a typical *month*? _____

(Oily fish=salmon, mackerel, sardines, trout, kippers)

4) How many portions of other (white) fish do you eat in a typical *month*?

_____

5) Have you purposefully changed your eating habits and diet in the past couple of years to include more oily fish? Yes • No •

6) If yes how long ago did you start eating more oily fish? _____

7) Do you take an omega-3 supplement? Yes • No •

If yes, how often (days per week)? _____
If yes, for how long (months, years)? _____
If yes, which type of supplement is it?
Cod liver oil •
Fish oil •
High EPA (EPA enriched) •
High DHA (DHA enriched) •
Vegetarian (Alpha-linoleic acid) •
Not sure •

8) Do you regularly take a vitamin supplement(s)? Yes • No •

If yes, how often (days per week)? _____

If yes, for how long (months, years)? _____

If yes which type of supplement(s) is it?

_____

9) How many times a week do you exercise? _____

10) Were you breastfed? Yes • No • Don't know •

11) The next few questions are about what you eat on a typical day. Please think about your eating habits carefully as we want to get as accurate picture as we can of your dietary habits. Please respond honestly as the information you provide will add to the quality of this research. Please also be as specific as you can e.g. 'cornflakes with semi-skimmed milk and 2 slices white toast with jam and butter' instead of just 'cereal and toast' and 'chicken salad sandwich on brown bread' instead of just 'sandwich'. If you do not usually eat that meal, please say so.

What do you normally eat for breakfast?

_____

_____

What do you normally eat for lunch?

_____

_____

What do you normally eat for dinner?

_____

_____

What snacks do you have on a typical day (including through the day and after dinner)?

_____

_____

APPENDIX III: All analysed associations between serum n-3 PUFAs and behavioural outcomes

Table III.1. Associations between serum DHA and COMPASS, mood and CDB outcome measures.

	n	*β*	*p*	95% C.I.
Immediate Word Recall (no. correct)	217	0.12	0.892	-.087, 1.14
Delayed Word Recall (no. correct)		0.06	0.401	-0.35, 0.87
Simple RT		0.02	0.454	-9.09, 11.40
Choice RT		0.12	0.420	-12.74, 15.93
Choice RT (% accuracy)		0.07	0.344	-0.41, 1.15
4 Choice RT		0.04	0.268	-16.52, 28.20
4 Choice RT (% accuracy)		-0.03	0.663	-0.45, 0.29
Stroop (RT)	212	0.06	0.370	-24.31, 65.01
Stroop (% accuracy)		0.07	0.303	-0.16, 0.50
Verbal Fluency	217	-0.06	0.367	-3.15, 1.17
Numeric Working Memory (RT)	216	0.10	0.157	-16.70, 102.83
Numeric Working Memory (% accuracy)		-0.18*	0.010	-2.42, -0.34
Alphabetic Working Memory (RT)	214	-0.09	0.173	-277.99, 50.39
Alphabetic Working Memory (% accuracy)		0.03	0.646	-0.83, 1.33
Corsi Blocks Span	217	-0.09	0.185	-0.53, 0.10
3-back task (RT)		0.03	0.675	-148.09, 228.07
3-back task (% accuracy)		-0.03	0.625	-4.84, 2.91
Telephone Number task (RT)		0.15*	0.030	80.61, 1588.82
Telephone Number task (% accuracy)		-0.11	0.109	-11.91, 1.21
Word Recognition (RT)		0.02	0.762	-43.67, 59.57
Word Recognition (% accuracy)		0.05	0.510	-1.71, 3.43
Picture Recognition (RT)		-0.01	0.923	-64.91, 58.81
Picture Recognition (% accuracy)		0.09	0.214	-1.08, 4.80
Names-to-Faces Recall (no. correct)		0.11	0.099	-0.24, 2.80
Bond-Lader alert		0.00	0.993	-3.39, 3.42
Bond-Lader calm		-0.03	0.709	-3.95, 2.69
Bond-Lader content		-0.06	0.377	-5.04, 1.91
DASS stress		0.08	0.244	-0.79, 3.10
DASS anxiety		0.04	0.575	-0.91, 1.63
DASS depression		0.04	0.528	-1.24, 2.41
Serial 3 Subtractions (no. correct)	213	-0.07	0.338	-5.15, 1.62
Serial 7 Subtractions (no. correct)		-0.08	0.305	-3.88, 0.98
RVIP (RT)	202	-0.09	0.996	-8.76, 1.84
RVIP (% accuracy)		0.11	0.166	-22.18, 26.03
RVIP (false alarms)		0.05	0.474	-1.67, 3.27
Mental fatigue (/100)	213	-0.03	0.670	-4.53, 3.11

Table III.2. Associations between serum EPA and COMPASS, mood and CDB outcome measures.

	n	*β*	*p*	95% C.I.
Immediate Word Recall (no. correct)	216	0.23**	0.001	0.49, 1.79
Delayed Word Recall (no. correct)		0.04	0.512	-0.44, 0.88
Simple RT		0.03	0.307	-9.10, 13.18
Choice RT		-0.05	0.927	-20.69, 10.26
Choice RT (% accuracy)		0.06	0.372	-0.46, 1.21
4 Choice RT		-0.02	0.694	-27.08, 21.51
4 Choice RT (% accuracy)		0.09	0.205	-0.14, 0.66
Stroop (RT)	211	0.00	0.962	-47.03, 49.37
Stroop (% accuracy)		0.10	0.141	-0.08, 0.58
Verbal Fluency	216	0.04	0.527	-1.61, 3.15
Numeric Working Memory (RT)	215	0.02	0.780	-54.63, 72.64
Numeric Working Memory (% accuracy)		0.03*	0.632	-0.85, 1.40
Alphabetic Working Memory (RT)	213	-0.06	0.376	-259.91, 98.68
Alphabetic Working Memory (% accuracy)		0.16	0.018	0.25, 2.57
Corsi Blocks Span	216	0.04	0.601	-0.25, 0.44
3-back task (RT)		0.11	0.110	-37.94, 369.60
3-back task (% accuracy)		0.08	0.249	-1.78, 6.84
Telephone Number task (RT)		0.05	0.425	-491.71, ,1163.36
Telephone Number task (% accuracy)		0.04	0.605	-5.25, 8.99
Word Recognition (RT)		-0.11	0.122	-99.56, 11.81
Word Recognition (% accuracy)		0.03	0.646	-2.16, 3.47
Picture Recognition (RT)		-0.06	0.369	-98.17, 36.59
Picture Recognition (% accuracy)		0.15*	0.027	0.41, 6.71
Names-to-Faces Recall (no. correct)		0.20**	0.003	0.83, 4.10
Bond-Lader alert		0.10	0.142	-0.93, 6.44
Bond-Lader calm		0.06	0.378	-1.99, 5.21
Bond-Lader content		0.00	0.967	-3.85, 3.70
DASS stress		-0.04	0.584	-2.70, 1.52
DASS anxiety		-0.13	0.062	-2.66, 0.07
DASS depression		0.00	0.970	-1.94, 2.02
Serial 3 Subtractions (no. correct)	212	0.08	0.432	-1.57, 5.83
Serial 7 Subtractions (no. correct)		0.05	0.542	-1.66, 3.66
RVIP (RT)	201	-0.04	0.109	-7.39, 4.43
RVIP (% accuracy)		0.14	0.531	-0.22, 52.85
RVIP (false alarms)		-0.09	0.306	-4.40, 1.06
Mental fatigue (/100)	212	-0.06	0.332	-6.07, 2.40

Table III.3. Associations between serum Total n-3 PUFAs (ALA+DHA+EPA) and COMPASS mood and CDB outcome measures.

	n	*β*	*p*	95% C.I.
Immediate Word Recall (no. correct)	218	0.17*	0.014	0.09, 0.77
Delayed Word Recall (no. correct)		0.04	0.518	-0.23, 0.45
Simple RT		0.002	0.452	-5.64, 5.83
Choice RT		-0.20	0.599	-9.15, 6.89
Choice RT (% accuracy)		0.11	0.119	-0.09, 0.78
4 Choice RT		0.01	0.341	-11.39, 13.63
4 Choice RT (% accuracy)		0.06	0.377	-0.11, 0.30
Stroop (RT)	213	0.05	0.503	-16.39, 33.30
Stroop (% accuracy)		0.11	0.106	-0.03, 0.33
Verbal Fluency	218	-0.02	0.762	-1.42, 1.04
Numeric Working Memory (RT)	217	0.08	0.262	-14.17, 51.75
Numeric Working Memory (% accuracy)		-0.07	0.306	-0.89, 0.28
Alphabetic Working Memory (RT)	215	-0.10	0.159	-157.95, 25.96
Alphabetic Working Memory (% accuracy)		0.10	0.143	-0.15, 1.05
Corsi Blocks Span	218	-0.04	0.585	-0.23, 0.13
3-back task (RT)		0.07	0.278	-47.25, 163.40
3-back task (% accuracy)		0.08	0.224	-0.85, 3.61
Telephone Number task (RT)		0.14*	0.043	14.82, 860.51
Telephone Number task (% accuracy)		-0.07	0.304	-5.62, 1.76
Word Recognition (RT)		-0.05	0.452	-39.87, 17.83
Word Recognition (% accuracy)		0.05	0.472	-0.92, 1.97
Picture Recognition (RT)		-0.03	0.615	-43.63, 25.87
Picture Recognition (% accuracy)		0.16*	0.020	0.30, 3.58
Names-to-Faces Recall (no. correct)		0.14*	0.035	0.06, 1.76
Bond-Lader alert		0.07	0.322	-0.94, 2.86
Bond-Lader calm		0.03	0.639	-1.42, 2.30
Bond-Lader content		-0.05	0.435	-2.72, 1.17
DASS stress		0.03	0.680	-0.86, 1.32
DASS anxiety		-0.04	0.526	-0.94, 0.48
DASS depression		0.03	0.712	-0.83, 1.22
Serial 3 Subtractions (no. correct)	214	-0.01	0.908	-2.05, 1.78
Serial 7 Subtractions (no. correct)		-0.03	0.799	-1.65, 1.09
RVIP (RT)		-0.05	0.361	-4.05, 1.91
RVIP (% accuracy)	203	0.08	0.366	-5.81, 21.10
RVIP (false alarms)		-0.04	0.727	-1.74, 1.03
Mental fatigue (/100)	214	-0.06	0.343	-3.06, 1.23

Table III.4. Associations between serum AA and COMPASS, mood and CDB outcome measures.

	n	*β*	*p*	95% C.I.
Immediate Word Recall (no. correct)	214	-0.24****	<0.001	-0.18, -0.05
Delayed Word Recall (no. correct)		-0.10	0.148	-0.11, 0.02
Simple RT		-0.09	0.187	-1.73, 0.34
Choice RT		0.01	0.869	-1.33, 1.57
Choice RT (% accuracy)		-0.09	0.202	-0.13, 0.03
4 Choice RT		-0.04	0.597	-2.87, 1.66
4 Choice RT (% accuracy)		-0.08	0.233	-0.06, 0.02
Stroop (RT)	209	-0.03	0.641	-4.42, 2.72
Stroop (% accuracy)		-0.19**	0.007	-0.08, -0.01
Verbal Fluency	215	-0.06	0.406	-0.19, 0.08
Numeric Working Memory (RT)	213	-0.08	0.241	-8.87, 2.24
Numeric Working Memory (% accuracy)		-0.03	0.625	-0.14, 0.08
Alphabetic Working Memory (RT)	211	-0.05	0.455	-33.56, 15.08
Alphabetic Working Memory (% accuracy)		-0.15*	0.029	-0.33, -0.02
Corsi Blocks Span	215	-0.04	0.520	-0.03, 0.01
3-back task (RT)	214	-0.09	0.196	-32.99, 6.81
3-back task (% accuracy)		-0.05	0.506	-0.32, 0.16
Telephone Number task (RT)		-0.07	0.315	-122.66, 39.71
Telephone Number task (% accuracy)		0.04	0.555	-0.49, 0.91
Word Recognition (RT)		0.00	0.997	-5.48, 5.46
Word Recognition (% accuracy)		-0.09	0.210	-0.25, 0.06
Picture Recognition (RT)	215	0.05	0.487	-2.39, 5.00
Picture Recognition (% accuracy)	214	-0.11	0.114	-0.56, 0.06
Names-to-Faces Recall (no. correct)		-0.18**	0.008	-0.38, -0.06
Bond-Lader alert		-0.13	0.059	-0.71, 0.01
Bond-Lader calm	215	0.03	0.640	-0.15, 0.25
Bond-Lader content		0.12	0.087	-0.03, 0.39
DASS stress		0.04	0.561	-0.08, 0.15
DASS anxiety		0.19**	0.005	0.03, 0.18
DASS depression		0.02	0.782	-0.09, 0.12
Serial 3 Subtractions (no. correct)	210	-0.08	0.253	-0.56, 0.15
Serial 7 Subtractions (no. correct)		-0.04	0.591	-0.32, 0.19
RVIP (RT)	199	0.00	0.995	-0.58, 0.58
RVIP (% accuracy)		-0.31	0.659	-3.08, 1.95
RVIP (false alarms)		0.12	0.086	-0.03, 0.50
Mental fatigue (/100)	210	0.17	0.013	0.102, 0.91

Table III.5. Associations between serum AA:EPA and COMPASS, mood and CDB outcome measures.

	n	*β*	*p*	95% C.I.
Immediate Word Recall (no. correct)	214	-0.24****	<0.001	-0.18, -0.05
Delayed Word Recall (no. correct)		-0.10	0.148	-0.11, 0.02
Simple RT		-0.09	0.187	-1.73, 0.34
Choice RT		0.01	0.869	-1.33, 1.57
Choice RT (% accuracy)		-0.09	0.202	-0.13, 0.03
4 Choice RT		-0.04	0.597	-2.87, 1.66
4 Choice RT (% accuracy)		-0.08	0.233	-0.06, 0.02
Stroop (RT)	209	-0.03	0.641	-4.42, 2.72
Stroop (% accuracy)		-0.19**	0.007	-0.08, -0.01
Verbal Fluency	215	-0.06	0.406	-0.19, 0.08
Numeric Working Memory (RT)	213	-0.08	0.241	-8.87, 2.24
Numeric Working Memory (% accuracy)		-0.03	0.625	-0.14, 0.08
Alphabetic Working Memory (RT)	211	-0.05	0.455	-33.56, 15.08
Alphabetic Working Memory (% accuracy)		-0.15*	0.029	-0.33, -0.02
Corsi Blocks Span	215	-0.04	0.520	-0.03, 0.01
3-back task (RT)	214	-0.09	0.196	-32.99, 6.81
3-back task (% accuracy)		-0.05	0.506	-0.32, 0.16
Telephone Number task (RT)		-0.07	0.315	-122.66, 39.71
Telephone Number task (% accuracy)		0.04	0.555	-0.49, 0.91
Word Recognition (RT)		0.00	0.997	-5.48, 5.46
Word Recognition (% accuracy)		-0.09	0.210	-0.25, 0.06
Picture Recognition (RT)	215	0.05	0.487	-2.39, 5.00
Picture Recognition (% accuracy)	214	-0.11	0.114	-0.56, 0.06
Names-to-Faces Recall (no. correct)		-0.18**	0.008	-0.38, -0.06
Bond-Lader alert		-0.13	0.059	-0.71, 0.01
Bond-Lader calm	215	0.03	0.640	-0.15, 0.25
Bond-Lader content		0.12	0.087	-0.03, 0.39
DASS stress		0.04	0.561	-0.08, 0.15
DASS anxiety		0.19**	0.005	0.03, 0.18
DASS depression		0.02	0.782	-0.09, 0.12
Serial 3 Subtractions (no. correct)	210	-0.08	0.253	-0.56, 0.15
Serial 7 Subtractions (no. correct)		-0.04	0.591	-0.32, 0.19
RVIP (RT)	199	0.00	0.995	-0.58, 0.58
RVIP (% accuracy)		-0.31	0.659	-3.08, 1.95
RVIP (false alarms)		0.12	0.086	-0.03, 0.50
Mental fatigue (/100)	210	0.17	0.013	0.102, 0.91

APPENDIX IV: Health and diet questionnaire utilised in Chapter 4

Age _____ **Glasses/Contacts Y/N _____**

Sex _____ **Handedness L/R _____**

Height _____ cm (to be completed by researcher)

Weight _____ kgs (to be completed by researcher)

1) How many portions of fruit and vegetables do you eat on a typical day? _____

(Juice only counts as 1 portion regardless is you drink multiple glasses)

2) How many portions of oily fish do you eat in a typical *month*? _____

(Oily fish=salmon, mackerel, sardines, trout, kippers)

3) How many portions of other (white) fish do you eat in a typical *month*? _____

4) Do you regularly take a vitamin supplement(s)? Yes • No •
If yes, how often (days per week)? _____
If yes, for how long (months, years)? _____
If yes which type of supplement(s) is it?
_____
_____

5) How many times a week do you exercise? _____

6) Were you breastfed? Yes • No • Don't know •

7) The next few questions are about what you eat on a typical day. Please think about your eating habits carefully as we want to get as accurate picture as we can of your dietary habits. Please respond honestly as the information you provide will add to the quality of this research. Please also be as specific as you can e.g. 'cornflakes with semi-skimmed milk and 2 slices white toast with jam and butter' instead of just 'cereal and toast' and 'chicken salad sandwich on brown bread' instead of just 'sandwich'. If you do not usually eat that meal, please say so.

What do you normally eat for breakfast?

_____

_____

What do you normally eat for lunch?

_____

What do you normally eat for dinner?

_____

_____

What snacks do you have on a typical day (including through the day and after dinner)?

_____

APPENDIX V: Family background questionnaire

Please answer the following questions about your family's socio-economic status (SES) as accurately as you can. If your household has only one parent/guardian, please only check the relevant box and leave the other blank. **Household refers to the home you grew up in.**

1) Which of these the highest level of education your parents/guardians have achieved?

	Father	Mother
16 Years	☐	☐
18 Years	☐	☐
Diploma level	☐	☐
Degree level	☐	☐
Postgraduate qualification	☐	☐

2) Which of these best describes your parent's/guardian's occupation?

	Father	Mother
Professional	☐	☐
Managerial/technical	☐	☐
Non-manual skilled	☐	☐
Partly skilled	☐	☐
Unskilled	☐	☐
Unemployed/unable to work	☐	☐
In full-time education	☐	☐
Retired	☐	☐

3) What is the approximate annual income of your household?

Under £10000	☐
£10000-£20000	☐
£20000-£30000	☐
£30000-£40000	☐
£40000-£50000	☐
£50000-£60000	☐
£60000-£70000	☐
£70000-£80000	☐
£80000-£90000	☐
£90000-£100000	☐
£100000+	☐

1) **Which treatment do you think you received?**

Fish oil ☐

Placebo ☐

2) **Why?**

APPENDIX VI: Average scores by group on the cognitive tasks employed in Chapter 5

Cognitive task performance by treatment group. Means and SEM are presented.

		Mean	SEM
Stroop (% accuracy)	Placebo	98.81	0.42
	DHA-rich FO	98.25	0.55
	EPA-rich FO	98.79	0.46
Stroop (RT)	Placebo	666.43	29.35
	DHA-rich FO	674.33	36.26
	EPA-rich FO	721.88	39.94
TOL (planning time)	Placebo	2398.14	312.64
	DHA-rich FO	3024.29	198.53
	EPA-rich FO	3172.00	269.12
TOL (time to complete)	Placebo	7343.29	656.66
	DHA-rich FO	9046.57	763.81
	EPA-rich FO	8659.88	390.49
3-back (% accuracy)	Placebo	91.21	2.16
	DHA-rich FO	89.01	5.14
	EPA-rich FO	81.57	3.54
3-back (RT)	Placebo	1280.27	194.78
	DHA-rich FO	1305.92	162.48
	EPA-rich FO	1299.33	333.86
WCST (% accuracy)	Placebo	80.29	3.29
	DHA-rich FO	89.71	2.49
	EPA-rich FO	82.87	2.02
WCST (no. errors)	Placebo	19.71	4.65
	DHA-rich FO	10.33	1.98
	EPA-rich FO	15.88	3.07
WCST (time to complete)	Placebo	1451.43	250.62
	DHA-rich FO	1252.67	104.86
	EPA-rich FO	1404.75	77.93

APPENDIX VII: Resting baseline NIRS data collected in Chapter 6

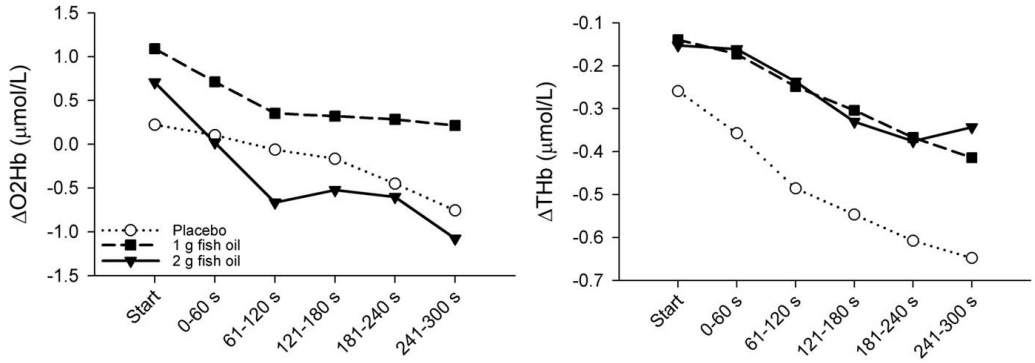

Figure VII.1. Resting baseline data for O2Hb and THb, by treatment group.

APPENDIX VIII: List of journal publications resulting from research conducted as part of this thesis

KENNEDY, D. O., JACKSON, P. A., ELLIOTT, J. M., SCHOLEY, A. B., ROBERTSON, B. C., GREER, J., TIPLADY, B., BUCHANAN, T. & HASKELL, C. F. (2009) Cognitive and mood effects of 8 weeks' supplementation with 400 mg or 1000 mg of the omega-3 essential fatty acid docosahexaenoic acid (DHA) in healthy children aged 10-12 years. *Nutritional Neuroscience,* 12**,** 48-56.

REFERENCES

Fish oil study's GCSE successes. (March 1, 2006). Retrieved April 28, 2006, from http://news.bbc.co.uk/1/hi/england/lancashire/4763720.stm

The Psychological Image Collection at Stirling (PICS). Retrieved February 20, 2007, from http://pics.psych.stir.ac.uk/.

Pupils test fish brain food pills. (April 5, 2006). Retrieved on April 28, 2006, from http://news.bbc.co.uk/1/hi/england/tees/4880552.stm.

(2010) COMMISSION REGULATION (EU) No 116/2010. *The Official Journal of the European Union,* L37**,** 16-18. Retrieved on March 10, 2010 from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:037:0016:0018:EN:PDF.

ABERG, M. A., ABERG, N., BRISMAN, J., SUNDBERG, R., WINKVIST, A. & TOREN, K. (2009) Fish intake of Swedish male adolescents is a predictor of cognitive performance. *Acta Paediatrica,* 98**,** 555-560.

ADAMS, P. B., LAWSON, S., SANIGORSKI, A. & SINCLAIR, A. J. (1996) Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids,* 31 Suppl**,** S157-61.

AGOSTONI, C., RIVA, E., TROJAN, S., BELLU, R. & GIOVANNINI, M. (1995) Docosahexaenoic Acid Status and Developmental Quotient of Healthy Term Infants. *Lancet,* 346**,** 638-638.

AGOSTONI, C., TROJAN, S., BELLU, R., RIVA, E., BRUZZESE, M. G. & GIOVANNINI, M. (1997) Developmental quotient at 24 months and fatty acid composition of diet in early infancy: A follow up study. *Archives of Disease in Childhood,* 76**,** 421-424.

AID, S., VANCASSEL, S., POUMES-BALLIHAUT, C., CHALON, S., GUESNET, P. & LAVIALLE, M. (2003) Effect of a diet-induced n-3 PUFA depletion on cholinergic parameters in the rat hippocampus. *Journal of Lipid Research,* 44**,** 1545-1551.

AKBAR, M., CALDERON, F., WEN, Z. & KIM, H. Y. (2005) Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc Natl Acad Sci U S A,* 102**,** 10858-63.

ALLARD, J. P., KURIAN, R., AGHDASSI, E., MUGGLI, R. & ROYALL, D. (1997) Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids,* 32**,** 535-541.

AMMINGER, G. P., BERGER, G. E., SCHAFER, M. R., KLIER, C., FRIEDRICH, M. H. & FEUCHT, M. (2007) Omega-3 Fatty Acids Supplementation in Children with Autism: A Double-blind Randomized, Placebo-controlled Pilot Study. *Biol Psychiatry,* 61**,** 551-3.

ANDERSON, C. M., TEICHER, M. H., POLCARI, A. & RENSHAW, P. F. (2002) Abnormal T2 relaxation time in the cerebellar vermis of adults sexually abused in childhood: potential role of the vermis in stress-enhanced risk for drug abuse. *Psychoneuroendocrinology,* 27**,** 231-244.

ANDERSON, G. J., NEURINGER, M., LIN, D. S. & CONNOR, W. E. (2005) Can prenatal n-3 fatty acid deficiency be completely reversed after birth? Effects on retinal and brain biochemistry and visual function in rhesus monkeys. *Pediatric Research,* 58**,** 865-872.

ANDERSON, I. M., NUTT, D. J. & DEAKIN, J. F. W. (2000) Evidence-based guidelines for treating depressive disorders with antidepressants: a revision of the 1993 British Association for Psychopharmacology guidelines. *Journal of Psychopharmacology,* 14**,** 3-20.

ANTYPA, N., VAN DER DOES, A. J. W., SMELT, A. H. M. & ROGERS, R. D. (2009) Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers. *Journal of Psychopharmacology,* 23**,** 831-840.

APA (2000) *Diagnostic and Statistical Manual of Mental Disorders IV,* Washington, DC, American Psychiatric Press Inc.

APPLETON, K. M., GUNNELL, D., PETERS, T. J., NESS, A. R., KESSLER, D. & ROGERS, P. J. (2008) No clear evidence of an association between plasma concentrations of n-3 long-chain polyunsaturated fatty acids and depressed mood in a non-clinical population. *Prostaglandins Leukot Essent Fatty Acids,* 78**,** 337-42.

APPLETON, K. M., HAYWARD, R. C., GUNNELL, D., PETERS, T. J., ROGERS, P. J., KESSLER, D. & NESS, A. R. (2006) Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials. *American Journal of Clinical Nutrition,* 84**,** 1308-1316.

ARMAH, C. K., JACKSON, K. G., DOMAN, I., JAMES, L., CHEGHANI, F. & MINIHANE, A. M. (2008) Fish oil fatty acids improve postprandial vascular reactivity in healthy men. *Clinical Science,* 114**,** 679-686.

ARTERBURN, L. M., BOSWELL, K. D., HENWOOD, S. M. & KYLE, D. J. (2000) A developmental safety study in rats using DHA- and ARA-rich single-cell oils. *Food and Chemical Toxicology,* 38**,** 763-771.

ASA (2006) Advertising Standards Authority Non-broadcast Adjudication: Dairy Crest Ltd. (June 21, 2006). Retrieved January 15, 2010 from http://www.asa.org.uk/Complaints-and-ASA-action/Adjudications/2006/6/Dairy-Crest-Ltd/CS_41426.aspx.

ASA (2007) Advertising Standards Adjudication on Potters Ltd. (December 12, 2007). Retrieved January 15, 2010 from http://www.asa.org.uk/Complaints-and-ASA-action/Adjudications/2007/12/Potters-Ltd/TF_ADJ_43685.aspx.

ASSIES, J., LIEVERSE, R., VREKEN, P., WANDERS, R. J., DINGEMANS, P. M. & LINSZEN, D. H. (2001) Significantly reduced docosahexaenoic and docosapentaenoic acid concentrations in erythrocyte membranes from

schizophrenic patients compared with a carefully matched control group. *Biol Psychiatry,* 49**,** 510-22.

AUESTAD, N., HALTER, R., HALL, R. T., BLATTER, M., BOGLE, M. L., BURKS, W., ERICKSON, J. R., FITZGERALD, K. M., DOBSON, V., INNIS, S. M., SINGER, L. T., MONTALTO, M. B., JACOBS, J. R., QIU, W. & BORNSTEIN, M. H. (2001) Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics,* 108**,** 372-381.

AUESTAD, N., MONTALTO, M. B., HALL, R. T., FITZGERALD, K. M., WHEELER, R. E., CONNOR, W. E., NEURINGER, M., CONNOR, S. L., TAYLOR, J. A. & HARTMANN, E. E. (1997) Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. *Pediatric Research,* 41**,** 1-10.

AWH, E., JONIDES, J., SMITH, E. E., SCHUMACHER, E. H., KOEPPE, R. A. & KATZ, S. (1996) Dissociation of storage and rehearsal in verbal working memory: Evidence from positron emission tomography. *Psychological Science,* 7**,** 25-31.

BAGGA, D., WANG, L., FARIAS-EISNER, R., GLASPY, J. A. & REDDY, S. T. (2003) Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proceedings of the National Academy of Sciences of the United States of America,* 100**,** 1751-1756.

BANG, H. O., DYERBERG, J. & HJORNE, N. (1976) Composition of Food Consumed by Greenland Eskimos. *Acta Medica Scandinavica,* 200**,** 69-73.

BARBERGER-GATEAU, P., LETENNEUR, L., DESCHAMPS, V., PERES, K., DARTIGUES, J. F. & RENAUD, S. (2002) Fish, meat, and risk of dementia: cohort study. *British Medical Journal,* 325**,** 932-+.

BARBERGER-GATEAU, P., RAFFAITIN, C., LETENNEUR, L., BERR, C., TZOURIO, C., DARTIGUES, J. F. & ALPEROVITCH, A. (2007) Dietary patterns and risk of dementia: the Three-City cohort study. *Neurology,* 69**,** 1921-30.

BARCELO-COBLIJN, G., KITAJKA, K., PUSKAS, L. G., HOGYES, E., ZVARA, A., HACKLER, L., JR. & FARKAS, T. (2003) Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio. *Biochim Biophys Acta,* 1632**,** 72-9.

BARTUS, R. T. (2000) On neurodegenerative diseases, models, and treatment strategies: Lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Experimental Neurology,* 163**,** 495-529.

BAZAN, N. G. (2006) The onset of brain injury and neurodegeneration triggers the synthesis of docosanoid neuroprotective signaling. *Cellular and Molecular Neurobiology,* 26**,** 901-913.

BEKAROGLU, M., ASLAN, Y., GEDIK, Y., DEGER, O., MOCAN, H., ERDURAN, E. & KARAHAN, C. (1996) Relationships between serum free fatty acids and

zinc, and attention deficit hyperactivity disorder: A research note. *Journal of Child Psychology and Psychiatry and Allied Disciplines,* 37**,** 225-227.

BELANGER, S. A., VANASSE, M., SPAHIS, S., SYLVESTRE, M. P., LIPPE, S., L'HEUREUX, F., GHADIRIAN, P., VANASSE, C. M. & LEVY, E. (2009) Omega-3 fatty acid treatment of children with attention-deficit hyperactivity disorder: A randomized, double-blind, placebo-controlled study. *Paediatrics & Child Health,* 14**,** 89-98.

BELL, J. G., MACKINLAY, E. E., DICK, J. R., MACDONALD, D. J., BOYLE, R. M. & GLEN, A. C. A. (2004) Essential fatty acids and phospholipase A(2) in autistic spectrum disorders. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 71**,** 201-204.

BELL, J. G., SARGENT, J. R., TOCHER, D. R. & DICK, J. R. (2000) Red blood cell fatty acid compositions in a patient with autistic spectrum disorder: a characteristic abnormality in neurodevelopmental disorders? *Prostaglandins Leukotrienes and Essential Fatty Acids,* 63**,** 21-25.

BELLUZZI, A., BOSCHI, S., BRIGNOLA, C., MUNARINI, A., CARIANI, G. & MIGLIO, F. (2000) Polyunsaturated fatty acids and inflammatory bowel disease. *American Journal of Clinical Nutrition,* 71**,** 339S-342S.

BEYDOUN, M. A., KAUFMAN, J. S., SATIA, J. A., ROSAMOND, W. & FOLSOM, A. R. (2007) Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr,* 85**,** 1103-11.

BHUGRA, D. (2005) The global prevalence of schizophrenia. *Plos Medicine,* 2**,** 372-373.

BIRCH, E. E., BIRCH, D. G., HOFFMAN, D. R. & UAUY, R. (1992) Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci,* 33**,** 3242-53.

BIRCH, E. E., GARFIELD, S., CASTANEDA, Y., HUGHBANKS-WHEATON, D., UAUY, R. & HOFFMAN, D. (2007) Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Human Development,* 83**,** 279-284.

BIRCH, E. E., GARFIELD, S., HOFFMAN, D. R., UAUY, R. & BIRCH, D. G. (2000) A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Developmental Medicine and Child Neurology,* 42**,** 174-181.

BIRCH, E. E., HOFFMAN, D. R., UAUY, R., BIRCH, D. G. & PRESTIDGE, C. (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatric Research,* 44**,** 201-209.

BLANK, C., NEUMANN, M. A., MAKRIDES, M. & GIBSON, R. A. (2002) Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. *J Lipid Res,* 43**,** 1537-1543.

BOND, A. & LADER, M. (1974) Use of Analog Scales in Rating Subjective Feelings. *British Journal of Medical Psychology,* 47**,** 211-218.

BONOCZK, P., PANCZEL, G. & NAGY, Z. (2002) Vinpocetine increases cerebral blood flow and oxygenation in stroke patients: a near infrared spectroscopy and transcranial Doppler study. *Eur J Ultrasound,* 15**,** 85-91.

BOUDRAULT, C., BAZINET, R. P. & MA, D. W. L. (2009) Experimental models and mechanisms underlying the protective effects of n-3 polyunsaturated fatty acids in Alzheimer's disease. *Journal of Nutritional Biochemistry,* 20**,** 1-10.

BOUNTZIOUKA, V., POLYCHRONOPOULOS, E., ZEIMBEKIS, A., PAPAVENETIOU, E., LADOUKAKI, E., PAPAIRAKLEOUS, N., GOTSIS, E., METALLINOS, G., LIONIS, C. & PANAGIOTAKOS, D. (2009) Long-term fish intake is associated with less severe depressive symptoms among elderly men and women: the MEDIS (MEDiterranean ISlands Elderly) epidemiological study. *J Aging Health,* 21**,** 864-80.

BOURRE, J. M. (2005) [Omega-3 fatty acids in psychiatry]. *Med Sci (Paris),* 21**,** 216-21.

BOURRE, J. M., DINH, L., BOITHIAS, C., DUMONT, O., PICIOTTI, M. & CUNNANE, S. (1997) Possible role of the choroid plexus in the supply of brain tissue with polyunsaturated fatty acids. *Neuroscience Letters,* 224**,** 1-4.

BROWNE, J. C., SCOTT, K. M. & SILVERS, K. M. (2006) Fish consumption in pregnancy and omega-3 status after birth are not associated with postnatal depression. *J Affect Disord,* 90**,** 131-9.

BRUNNER, E. J., JONES, P. J. S., FRIEL, S. & BARTLEY, M. (2009) Fish, human health and marine ecosystem health: policies in collision. *International Journal of Epidemiology,* 38**,** 93-100.

BUNCE, S. C., IZZETOGLU, M., IZZETOGLU, K., ONARAL, B. & POURREZAEI, K. (2006) Functional near-infrared spectroscopy - An emerging neuroimaging modality. *Ieee Engineering in Medicine and Biology Magazine,* 25**,** 54-62.

BURDGE, G. C. & CALDER, P. C. (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev,* 45**,** 581-97.

BURDGE, G. C., FINNEGAN, Y. E., MINIHANE, A. M., WILLIAMS, C. M. & WOOTTON, S. A. (2003) Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [C-13]alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. *British Journal of Nutrition,* 90**,** 311-321.

BURDGE, G. C., JONES, A. E. & WOOTTON, S. A. (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *British Journal of Nutrition,* 88**,** 355-363.

BURGESS, J. R., STEVENS, L., ZHANG, W. & PECK, L. (2000) Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. *Am J Clin Nutr,* 71**,** 327S-30S.

BUYDENS-BRANCHEY, L. & BRANCHEY, M. (2006) n-3 Polyunsaturated fatty acids decrease anxiety feelings in a population of substance abusers. *Journal of Clinical Psychopharmacology,* 26**,** 661-665.

CALDER, P. C. (2006a) n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition,* 83**,** 1505S-1519S.

CALDER, P. C. (2006b) Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids,* 75**,** 197-202.

CALDER, P. C. (2007) Immunomodulation by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids,* 77**,** 327-35.

CARLSON, S. E., FORD, A. J., WERKMAN, S. H., PEEPLES, J. M. & KOO, W. W. K. (1996) Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatric Research,* 39**,** 882-888.

CARRIE, I., CLEMENT, M., DE JAVEL, D., FRANCES, H. & BOURRE, J. M. (2000) Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. *Journal of Lipid Research,* 41**,** 473-480.

CARROLL, D., RING, C., SUTER, M. & WILLEMSEN, G. (2000) The effects of an oral multivitamin combination with calcium, magnesium, and zinc on psychological well-being in healthy young male volunteers: a double-blind placebo-controlled trial. *Psychopharmacology,* 150**,** 220-225.

CARVER, J. D., BENFORD, V. J., HAN, B. & CANTOR, A. B. (2001) The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Research Bulletin,* 56**,** 79-85.

CHALON, S., DELION-VANCASSEL, S., BELZUNG, C., GUILLOTEAU, D., LEGUISQUET, A. M., BESNARD, J. C. & DURAND, G. (1998) Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. *Journal of Nutrition,* 128**,** 2512-2519.

CHALON, S., VANCASSEL, S., ZIMMER, L., GUILLOTEAU, D. & DURAND, G. (2001) Polyunsaturated fatty acids and cerebral function: Focus on monoaminergic neurotransmission. *Lipids,* 36**,** 937-944.

CHANCE, B., LEIGH, J. S., MIYAKE, H., SMITH, D. S., NIOKA, S., GREENFELD, R., FINANDER, M., KAUFMANN, K., LEVY, W., YOUNG, M., COHEN, P., YOSHIOKA, H. & BORETSKY, R. (1988) Comparison of Time-Resolved and Time-Unresolved Measurements of Deoxyhemoglobin in Brain. *Proceedings of the National Academy of Sciences of the United States of America,* 85**,** 4971-4975.

CHERUBINI, A., ANDRES-LACUEVA, C., MARTIN, A., LAURETANI, F., IORIO, A. D., BARTALI, B., CORSI, A., BANDINELLI, S., MATTSON, M. P. & FERRUCCI, L. (2007) Low plasma N-3 fatty acids and dementia in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci,* 62**,** 1120-6.

CLANDININ, M. T., CHAPPELL, J. E., LEONG, S., HEIM, T., SWYER, P. R. & CHANCE, G. W. (1980a) Extra-Uterine Fatty-Acid Accretion in Infant Brain - Implications for Fatty-Acid Requirements. *Early Human Development,* 4**,** 131-138.

CLANDININ, M. T., CHAPPELL, J. E., LEONG, S., HEIM, T., SWYER, P. R. & CHANCE, G. W. (1980b) Intrauterine Fatty-Acid Accretion Rates in Human-Brain - Implications for Fatty-Acid Requirements. *Early Human Development,* 4**,** 121-129.

COLCOMBE, S. J., ERICKSON, K. I., RAZ, N., WEBB, A. G., COHEN, N. J., MCAULEY, E. & KRAMER, A. F. (2003) Aerobic fitness reduces brain tissue loss in aging humans. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences,* 58**,** 176-180.

COLQUHOUN, I. & BUNDAY, S. (1981) A Lack of Essential Fatty-Acids as a Possible Cause of Hyperactivity in Children. *Medical Hypotheses,* 7**,** 673-679.

CONKLIN, S. M., GIANAROS, P. J., BROWN, S. M., YAO, J. K., HARIRI, A. R., MANUCK, S. B. & MULDOON, M. F. (2007) Long-chain omega-3 fatty acid intake is associated positively with corticolimbic gray matter volume in healthy adults. *Neuroscience Letters,* 421**,** 209-212.

CONNOR, W. E. & NEURINGER, M. (1988) The effects of n-3 fatty acid deficiency and repletion upon the fatty acid composition and function of the brain and retina. *Prog Clin Biol Res,* 282**,** 275-94.

CONNOR, W. E., NEURINGER, M. & LIN, D. S. (1990) Dietary-Effects on Brain Fatty-Acid Composition - the Reversibility of N-3 Fatty-Acid Deficiency and Turnover of Docosahexaenoic Acid in the Brain, Erythrocytes, and Plasma of Rhesus-Monkeys. *Journal of Lipid Research,* 31**,** 237-247.

CONQUER, J. A., TIERNEY, M. C., ZECEVIC, J., BETTGER, W. J. & FISHER, R. H. (2000) Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids,* 35**,** 1305-12.

CYHLAROVA, E., BELL, J. G., DICK, J. R., MACKINLAY, E. E., STEIN, J. F. & RICHARDSON, A. J. (2007) Membrane fatty acids, reading and spelling in dyslexic and non-dyslexic adults. *Eur Neuropsychopharmacol,* 17**,** 116-21.

DA SILVA, A. X., LAVIALLE, F., GENDROT, G., GUESNET, P., ALESSANDRI, J. M. & LAVIALLE, M. (2002) Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. *Journal of Neurochemistry,* 81**,** 1328-1337.

DALTON, A., WOLMARANS, P., WITTHUHN, R. C., VAN STUIJVENBERG, M. E., SWANEVELDER, S. A. & SMUTS, C. M. (2009) A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. *Prostaglandins Leukot Essent Fatty Acids,* 80**,** 143-9.

DANGOUR, A. D., ALLEN, E., ELBOURNE, D., FLETCHER, A., RICHARDS, M. & UAUY, R. (2009) Fish Consumption and Cognitive Function among Older People in the UK: Baseline Data from the Opal Study. *J Nutr Health Aging,* 13**,** 198-202.

DANGOUR, A. D., CLEMENS, F., ELBOURNE, D., FASEY, N., FLETCHER, A. E., HARDY, P., HOLDER, G. E., HUPPERT, F. A., KNIGHT, R., LETLEY, L., RICHARDS, M., TRUESDALE, A., VICKERS, M. & UAUY, R. (2006) An randomised controlled trial investigation the effects of n-3 long-chain polyunsaturated fatty acid supplementation on cognitive and retinal function in cognitively healthy older people: the Older People And n-3 Long-chain polyunsaturated fatty acids (OPAL) study protocol. *Nutrition Journal,* 5.

DAS, U. N. (2007) Is depression a low-grade systemic inflammatory condition? *Am J Clin Nutr,* 85**,** 1665-6; author reply 1666.

DAS, U. N. (2008) Folic acid and polyunsaturated fatty acids improve cognitive function and prevent depression, dementia, and Alzheimer's disease - But how and why? *Prostaglandins Leukotrienes and Essential Fatty Acids,* 78**,** 11-19.

DAVIS, K. L., KAHN, R. S., KO, G. & DAVIDSON, M. (1991) Dopamine in Schizophrenia - a Review and Reconceptualization. *American Journal of Psychiatry,* 148**,** 1474-1486.

DE CATERINA, R. & BASTA, G. (2001) n-3 fatty acids and the inflammatory response - biological background. *European Heart Journal Supplements,* 3**,** D42-D49.

DE CATERINA, R., LIAO, J. K. & LIBBY, P. (2000) Fatty acid modulation of endothelial activation. *American Journal of Clinical Nutrition,* 71**,** 213S-223S.

DE FOCKERT, J. W. (2005) Keeping priorities: the role of working memory and selective attention in cognitive aging. *Sci Aging Knowledge Environ,* 2005**,** pe34.

DE GROOT, R. H. M., ADAM, J., JOLLES, J. & HORNSTRA (2004) Alpha-linolenic acid supplementation during human pregnancy does not effect cognitive functioning. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 70**,** 41-47.

DE LA PRESA OWENS, S. D. & INNIS, S. M. (1999) Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotoninergic neurotransmitters in frontal cortex caused by a linoleic and alpha-linolenic acid deficient diet in formula-fed piglets. *Journal of Nutrition,* 129**,** 2088-2093.

DE VRIESE, S. R., CHRISTOPHE, A. B. & MAES, M. (2003) Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sci,* 73**,** 3181-7.

DE WILDE, M. C., FARKAS, E., GERRITS, M., KILIAAN, A. J. & LUITEN, P. G. M. (2002) The effect of n-3 polyunsaturated fatty acid-rich diets on cognitive

and cerebrovascular parameters in chronic cerebral hypoperfusion. *Brain Research,* 947**,** PII S0006-8993(02)02695-1.

DE WILDE, M. C., HOGYES, E., KILIAAN, A. J., FARKAS, T., LUITEN, P. G. & FARKAS, E. (2003) Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats. *Brain Res,* 988**,** 9-19.

DEBUSK, R. M., FOGARTY, C. P., ORDOVAS, J. M. & KORNMAN, K. S. (2005) Nutritional genomics in practice: Where do we begin? *Journal of the American Dietetic Association,* 105**,** 589-598.

DEL PARIGI, A., PANZA, F., CAPURSO, C. & SOLFRIZZI, V. (2006) Nutritional factors, cognitive decline, and dementia. *Brain Research Bulletin,* 69**,** 1-19.

DELION, S., CHALON, S., GUILLOTEAU, D., BESNARD, J. C. & DURAND, G. (1996) alpha-linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. *Journal of Neurochemistry,* 66**,** 1582-1591.

DELION, S., CHALON, S., HERAULT, J., GUILLOTEAU, D., BESNARD, J. C. & DURAND, G. (1994) Chronic Dietary Alpha-Linolenic Acid Deficiency Alters Dopaminergic and Serotoninergic Neurotransmission in Rats. *Journal of Nutrition,* 124**,** 2466-2476.

DEPPE, M., RINGELSTEIN, E. B. & KNECHT, S. (2004) The investigation of functional brain lateralization by transcranial Doppler sonography. *Neuroimage,* 21**,** 1124-1146.

DINAN, T., SIGGINS, L., SCULLY, P., O'BRIEN, S., ROSS, P. & STANTON, C. (2009) Investigating the inflammatory phenotype of major depression: Focus on cytokines and polyunsaturated fatty acids. *Journal of Psychiatric Research,* 43**,** 471-476.

DROVER, J., HOFFMAN, D. R., CASTANEDA, Y. S., MORALE, S. E. & BIRCH, E. E. (2009) Three randomized controlled trials of early long-chain polyunsaturated Fatty Acid supplementation on means-end problem solving in 9-month-olds. *Child Dev,* 80**,** 1376-84.

DRUMMOND, S. P. A., BROWN, G. G., STRICKER, J. L., BUXTON, R. B., WONG, E. C. & GILLIN, J. C. (1999) Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport,* 10**,** 3745-3748.

DULLEMEIJER, C., DURGA, J., BROUWER, I. A., VAN DE REST, O. V., KOK, F. J., BRUMMER, R. J. M., VAN BOXTEL, M. P. J. & VERHOEF, P. (2007) n-3 Fatty acid proportions in plasma and cognitive performance in older adults. *Am J Clin Nutr,* 86**,** 1479-1485.

DUNSTAN, J. A., SIMMER, K., DIXON, G. & PRESCOTT, S. L. (2008) Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed,* 93**,** F45-50.

EDWARDS, R., PEET, M., SHAY, J. & HORROBIN, D. (1998) Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord,* 48**,** 149-55.

ENDRES, S., GHORBANI, R., KELLEY, V. E., GEORGILIS, K., LONNEMANN, G., VANDERMEER, J. W. M., CANNON, J. G., ROGERS, T. S., KLEMPNER, M. S., WEBER, P. C., SCHAEFER, E. J., WOLFF, S. M. & DINARELLO, C. A. (1989) The Effect of Dietary Supplementation with N-3 Poly-Unsaturated Fatty-Acids on the Synthesis of Interleukin-1 and Tumor Necrosis Factor by Mononuclear-Cells. *New England Journal of Medicine,* 320**,** 265-271.

ENGELHART, M. J., GEERLINGS, M. I., RUITENBERG, A., VAN SWIETEN, J. C., HOFMAN, A., WITTEMAN, J. C. & BRETELER, M. M. (2002) Diet and risk of dementia: Does fat matter?: The Rotterdam Study. *Neurology,* 59**,** 1915-21.

ENGSTROM, K., SALDEEN, A. S., YANG, B., MEHTA, J. L. & SALDEEN, T. (2009) Effect of fish oils containing different amounts of EPA, DHA, and antioxidants on plasma and brain fatty acids and brain nitric oxide synthase activity in rats. *Ups J Med Sci,* 114**,** 206-13.

ESFA (2009) Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *The EFSA Journal,* 1176**,** 1-11. Retrived on March 10, 2010 from http://www.efsa.europa.eu/en/scdocs/doc/1176.pdf.

FALLGATTER, A. J. & STRIK, W. K. (1997) Right frontal activation during the continuous performance test assessed with near-infrared spectroscopy in healthy subjects. *Neuroscience Letters,* 223**,** 89-92.

FALLGATTER, A. J. & STRIK, W. K. (1998) Frontal brain activation during the Wisconsin Card Sorting Test assessed with two-channel near-infrared spectroscopy. *European Archives of Psychiatry and Clinical Neuroscience,* 248**,** 245-249.

FARKAS, E., DE WILDE, M. C., KILIAAN, A. J. & LUITEN, P. G. M. (2002) Chronic cerebral hypoperfusion-related neuropathologic changes and compromised cognitive status: Window of treatment. *Drugs of Today,* 38**,** 365-376.

FARKAS, E. & LUITEN, P. G. M. (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Progress in Neurobiology,* 64**,** 575-611.

FAROOQUI, A. A., YANG, H. C., ROSENBERGER, T. A. & HORROCKS, L. A. (1997) Phospholipase A(2) and its role in brain tissue. *Journal of Neurochemistry,* 69**,** 889-901.

FARQUHARSON, J., COCKBURN, F., PATRICK, W. A., JAMIESON, E. C. & LOGAN, R. W. (1993) Effect of Diet on Infant Subcutaneous Tissue Triglyceride Fatty-Acids. *Archives of Disease in Childhood,* 69**,** 589-593.

FEDOROVA, I. & SALEM, N., JR. (2006) Omega-3 fatty acids and rodent behavior. *Prostaglandins Leukot Essent Fatty Acids,* 75**,** 271-89.

FELLER, S. E., GAWRISCH, K. & MACKERELL, A. D. (2002) Polyunsaturated fatty acids in lipid bilayers: Intrinsic and environmental contributions to their

unique physical properties. *Journal of the American Chemical Society,* 124**,** 318-326.

FENTON, W. S., DICKERSON, F., BORONOW, J., HIBBELN, J. R. & KNABLE, M. (2001) A placebo-controlled trial of omega-3 fatty acid (ethyl eicosapentaenoic acid) supplementation for residual symptoms and cognitive impairment in schizophrenia. *American Journal of Psychiatry,* 158**,** 2071-2074.

FERNSTROM, J. D. (2000) Can nutrient supplements modify brain function? *American Journal of Clinical Nutrition,* 71**,** 1669S-1673S.

FERRUCCI, L., CHERUBINI, A., BANDINELLI, S., BARTALI, B., CORSI, A., LAURETANI, F., MARTIN, A., ANDRES-LACUEVA, C., SENIN, U. & GURALNIK, J. M. (2006) Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab,* 91**,** 439-46.

FITZGERALD, P. B., SRITHIRAN, A., BENITEZ, J., DASKALAKIS, Z. Z., OXLEY, T. J., KULKARNI, J. & EGAN, G. F. (2008) An fMRI study of prefrontal brain activation during multiple tasks in patients with major depressive disorder. *Human Brain Mapping,* 29**,** 490-501.

FLEITH, M. & CLANDININ, M. T. (2005) Dietary PUFA for preterm and term infants: Review of clinical studies. *Critical Reviews in Food Science and Nutrition,* 45**,** 205-229.

FONTANI, G., CORRADESCHI, A., FELICI, F., ALFATTI, S., MIGLIORI, S. & LODI, L. (2005) Cognitive and physiological effects of Omega-3 polyunsaturated fatty acid supplementation in healthy subjects. *Eur J Clin Invest,* 35**,** 691-699.

FOTUHI, M., MOHASSEL, P. & YAFFE, K. (2009) Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association. *Nature Clinical Practice Neurology,* 5**,** 140-152.

FRANGOU, S., LEWIS, M. & MCCRONE, P. (2006) Efficacy of ethyl-eicosapentaenoic acid in bipolar depression: randomised double-blind placebo-controlled study. *British Journal of Psychiatry,* 188**,** 46-50.

FRASER, T., TAYLER, H. & LOVE, S. (2009) Fatty Acid Composition of Frontal, Temporal and Parietal Neocortex in the Normal Human Brain and in Alzheimer's Disease. *Neurochem Res*.

FREEMAN, M. P. (2006) Omega-3 fatty acids and perinatal depression: A review of the literature and recommendations for future research. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 75**,** 291-297.

FREEMAN, M. P., DAVIS, M., SINHA, P., WISNER, K. L., HIBBELN, J. R. & GELENBERG, A. J. (2008) Omega-3 fatty acids and supportive psychotherapy for perinatal depression: A randomized placebo-controlled study. *J Affect Disord*.

FREEMAN, M. P., HIBBELN, J. R., WISNER, K. L., BRUMBACH, B. H., WATCHMAN, M. & GELENBERG, A. J. (2006a) Randomized dose-ranging

pilot trial of omega-3 fatty acids for postpartum depression. *Acta Psychiatrica Scandinavica,* 113**,** 31-35.

FREEMAN, M. P., HIBBELN, J. R., WISNER, K. L., DAVIS, J. M., MISCHOULON, D., PEET, M., KECK, P. E., MAYANGELL, L. B., RICHARDSON, A. J., LAKE, J. & STOLL, A. L. (2006b) Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *Journal of Clinical Psychiatry,* 67**,** 1954-1967.

FREUND-LEVI, Y., BASUN, H., CEDERHOLM, T., FAXEN-IRVING, G., GARLIND, A., GRUT, M., VEDIN, I., PALMBLAD, J., WAHLUND, L. O. & ERIKSDOTTER-JONHAGEN, M. (2007) Omega-3 supplementation in mild to moderate Alzheimer's disease: effects on neuropsychiatric symptoms. *Int J Geriatr Psychiatry*.

FREUND-LEVI, Y., BASUN, H., CEDERHOLM, T., FAXEN-IRVING, G., GARLIND, A., GRUT, M., VEDIN, I., PALMBLAD, J., WAHLUND, L. O. & ERIKSDOTTER-JONHAGEN, M. (2008) Omega-3 supplementation in mild to moderate Alzheimer's disease: effects on neuropsychiatric symptoms. *International Journal of Geriatric Psychiatry,* 23**,** 161-169.

FREUND-LEVI, Y., ERIKSDOTTER-JONHAGEN, M., CEDERHOLM, T., BASUN, H., FAXEN-IRVING, G., GARLIND, A., VEDIN, I., VESSBY, B., WAHLUND, L. O. & PALMBLAD, J. (2006) omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study - A randomized double-blind trial. *Archives of Neurology,* 63**,** 1402-+.

FUX, M., BENJAMIN, J. & NEMETS, B. (2004) A placebo-controlled cross-over trial of adjunctive EPA in OCD. *J Psychiatr Res,* 38**,** 323-5.

GAMOH, S., HASHIMOTO, M., HOSSAIN, S. & MASUMURA, S. (2001) Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. *Clinical and Experimental Pharmacology and Physiology,* 28**,** 266-270.

GAMOH, S., HASHIMOTO, M., SUGIOKA, K., HOSSAIN, M. S., HATA, N., MISAWA, Y. & MASUMURA, S. (1999) Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats. *Neuroscience,* 93**,** 237-241.

GELEIJNSE, J. M., GILTAY, E. J., GROBBEE, D. E., DONDERS, A. R. T. & KOK, F. J. (2002) Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *Journal of Hypertension,* 20**,** 1493-1499.

GERSTER, H. (1998) Can adults adequately convert alpha-linolenic acid (18 : 3n-3) to eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic acid (22 : 6n-3)? *International Journal for Vitamin and Nutrition Research,* 68**,** 159-173.

GIBNEY, M. J. & HUNTER, B. (1993) The Effects of Short-Term and Long-Term Supplementation with Fish Oil on the Incorporation of N-3 Polyunsaturated Fatty-Acids into Cells of the Immune-System in Healthy-Volunteers. *European Journal of Clinical Nutrition,* 47**,** 255-259.

GIEM, P., BEESON, W. L. & FRASER, G. E. (1993) The Incidence of Dementia and Intake of Animal Products - Preliminary Findings from the Adventist Health Study. *Neuroepidemiology,* 12**,** 28-36.

GILLETTE, S. (2009) The Multidomain Alzheimer Preventive Trial (MAPT): A new approach for the prevention of Alzheimer's disease. *Alzheimer's and Dementia,* 5**,** P145-P145.

GILLUM, R. F., MUSSOLINO, M. & MADANS, J. H. (2000) The relation between fish consumption, death from all causes, and incidence of coronary heart disease: The NHANES I Epidemiologic Follow-up Study. *Journal of Clinical Epidemiology,* 53**,** 237-244.

GRAEFF, F. G., GUIMARAES, F. S., DEANDRADE, T. & DEAKIN, J. F. W. (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacology Biochemistry and Behavior,* 54**,** 129-141.

GREINER, R. S., MORIGUCHI, T., HUTTON, A., SLOTNICK, B. M. & SALEM, N. (1999) Rats with low levels of brain docosahexaenoic acid show impaired performance in olfactory-based and spatial learning tasks. *Lipids,* 34**,** S239-S243.

GREINER, R. S., MORIGUCHI, T., SLOTNICK, B. M., HUTTON, A. & SALEM, N. (2001) Olfactory discrimination deficits in n-3 fatty acid-deficient rats. *Physiol Behav,* 72**,** 379-85.

GRONN, M., CHRISTENSEN, E., HAGVE, T. A. & CHRISTOPHERSEN, B. O. (1991) Peroxisomal Retroconversion of Docosahexaenoic Acid (22-6(N-3)) to Eicosapentaenoic Acid (20-5(N-3)) Studied in Isolated Rat-Liver Cells. *Biochimica Et Biophysica Acta,* 1081**,** 85-91.

HAGGARTY, P. (2004) Effect of placental function on fatty acid requirements during pregnancy. *European Journal of Clinical Nutrition,* 58**,** 1559-1570.

HAMAZAKI, T. & HIRAYAMA, S. (2004) The effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder - a placebo-controlled double-blind study. *European Journal of Clinical Nutrition,* 58**,** 838-838.

HAMAZAKI, T., ITOMURA, M., SAWAZAKI, S. & NAGAO, Y. (1999) Anti-stress effects of DHA. *2nd International Conference on Food Factors (2nd ICoFF).* Kyoto, Japan, Ios Press.

HAMAZAKI, T., SAWAZAKI, S., ITOMURA, M., ASAOKA, E., NAGAO, Y., NISHIMURA, N., YAZAWA, K., KUWAMORI, T. & KOBAYASHI, M. (1996) The effect of docosahexaenoic acid on aggression in young adults. A placebo-controlled double-blind study. *J Clin Invest,* 97**,** 1129-33.

HAMAZAKI, T., SAWAZAKI, S., NAGAO, Y., KUWAMORI, T., YAZAWA, K., MIZUSHIMA, Y. & KOBAYASHI, M. (1998) Docosahexaenoic acid does not affect aggression of normal volunteers under nonstressful conditions. A randomized, placebo-controlled, double-blind study. *Lipids,* 33**,** 663-7.

HAMILTON, L., GREINER, R., SALEM, N., JR. & KIM, H. Y. (2000) n-3 fatty acid deficiency decreases phosphatidylserine accumulation selectively in neuronal tissues. *Lipids,* 35**,** 863-9.

HARRIS, W. S. (2007) International recommendations for consumption of long-chain omega-3 fatty acids. *J Cardiovasc Med (Hagerstown),* 8 Suppl 1**,** S50-2.

HARRIS, W. S., MOZAFFARIAN, D., LEFEVRE, M., TONER, C. D., COLOMBO, J., CUNNANE, S. C., HOLDEN, J. M., KLURFELD, D. M., MORRIS, M. C. & WHELAN, J. (2009) Towards Establishing Dietary Reference Intakes for Eicosapentaenoic and Docosahexaenoic Acids. *J Nutr*.

HARRIS, W. S., RAMBJOR, G. S., WINDSOR, S. L. & DIEDERICH, D. (1997) n-3 fatty acids and urinary excretion of nitric oxide metabolites in humans. *American Journal of Clinical Nutrition,* 65**,** 459-464.

HARRIS, W. S. & VON SCHACKY, C. (2004) The Omega-3 Index: a new risk factor for death from coronary heart disease? *Preventive Medicine,* 39**,** 212-220.

HASHIMOTO, M., HOSSAIN, M. S., YAMASAKI, H., YAZAWA, K. & MASUMURA, S. (1999) Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. *Lipids,* 34**,** 1297-1304.

HASHIMOTO, M., HOSSAIN, S., AGDUL, H. & SHIDO, O. (2005a) Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids,* 1738**,** 91-98.

HASHIMOTO, M., HOSSAIN, S., SHIMADA, T., SUGIOKA, K., YAMASAKI, H., FUJII, Y., ISHIBASHI, Y., OKA, J. I. & SHIDO, O. (2002) Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *Journal of Neurochemistry,* 81**,** 1084-1091.

HASHIMOTO, M., HOSSAIN, S., TANABE, Y., KAWASHIMA, A., HARADA, T., YANO, T., MIZUGUCHI, K. & SHIDO, O. (2008) The protective effect of dietary eicosapentaenoic acid against impairment of spatial cognition learning ability in rats infused with amyloid beta((1-40)). *J Nutr Biochem*.

HASHIMOTO, M., TANABE, Y., FUJII, Y., KIKUTA, T., SHIBATA, H. & SHIDO, O. (2005b) Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. *Journal of Nutrition,* 135**,** 549-555.

HASKELL, C. F. & KENNEDY, D. O. (In preparation) Haemodynamic and behavioural effects of caffeine in habitual and non-habitual consumers of caffeine: A Near Infrared Spectroscopy study.

HASKELL, C. F., KENNEDY, D. O., WESNES, K. A. & SCHOLEY, A. B. (2005) Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. *Psychopharmacology,* 179**,** 813-825.

HASKELL, C. F., SCHOLEY, A. B., JACKSON, P. A., ELLIOTT, J. M., DEFEYTER, M. A., GREER, J., ROBERTSON, B. C., BUCHANAN, T., TIPLADY, B. & KENNEDY, D. O. (2008) Cognitive and mood effects in healthy children during 12 weeks' supplementation with multi-vitamin/minerals. *Br J Nutr,* 100**,** 1086-96.

HAWKINS, J. D. (1991) *Gene structure and expression,* Cambridge, Cambridge University Press.

HELLAND, I. B., SMITH, L., BLOMEN, B., SAAREM, K., SAUGSTAD, O. D. & DREVON, C. A. (2008) Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics,* 122**,** e472-9.

HELLAND, I. B., SMITH, L., SAAREM, K., SAUGSTAD, O. D. & DREVON, C. A. (2003) Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics,* 111**,** e39-44.

HENDERSON, L., GREGORY, J. & SWAN, G. (2002) The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed. TSO, UK.

HERSHEY, T., BLACK, K. J., HARTLEIN, J., BRAVER, T. S., BARCH, D. A., CARL, J. L. & PERLMUTTER, J. S. (2004) Dopaminergic modulation of response inhibition: an fMRI study. *Cognitive Brain Research,* 20**,** 438-448.

HEUDE, B., DUCIMETIERE, P. & BERR, C. (2003) Cognitive decline and fatty acid composition of erythrocyte membranes - The EVA Study. *Am J Clin Nutr,* 77**,** 803-808.

HIBBELN, J. R. (1998) Fish consumption and major depression. *Lancet,* 351**,** 1213.

HIBBELN, J. R. (2002) Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. *J Affect Disord,* 69**,** 15-29.

HIBBELN, J. R., DAVIS, J. M., STEER, C., EMMETT, P., ROGERS, I., WILLIAMS, C. & GOLDING, J. (2007) Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet,* 369**,** 578-85.

HIBBELN, J. R., NIEMINEN, L. R., BLASBALG, T. L., RIGGS, J. A. & LANDS, W. E. (2006) Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. *Am J Clin Nutr,* 83**,** 1483S-1493S.

HIBBELN, J. R. & SALEM, N., JR. (1995) Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. *Am J Clin Nutr,* 62**,** 1-9.

HIMMELHEBER, A. M., SARTER, M. & BRUNO, J. P. (2000) Increases in cortical acetylcholine release during sustained attention performance in rats. *Cognitive Brain Research,* 9**,** 313-325.

HIRASHIMA, F., PAROW, A. M., STOLL, A. L., DEMOPULOS, C. M., DAMICO, K. E., ROHAN, M. L., ESKESEN, J. G., ZUO, C. S., COHEN, B. M. & RENSHAW, P. F. (2004) Omega-3 fatty acid treatment and T-2 whole brain relaxation times in bipolar disorder. *American Journal of Psychiatry,* 161**,** 1922-1924.

HIRAYAMA, S., HAMAZAKI, T. & TERASAWA, K. (2004) Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder - a placebo-controlled double-blind study. *European Journal of Clinical Nutrition,* 58**,** 467-473.

HOGE, E. A., BRANDSTETTER, K., MOSHIER, S., POLLACK, M. H., WONG, K. K. & SIMON, N. M. (2009) Broad Spectrum of Cytokine Abnormalities in Panic Disorder and Posttraumatic Stress Disorder. *Depression and Anxiety,* 26**,** 447-455.

HOLICK, M. F. (2007) Vitamin D deficiency. *New England Journal of Medicine,* 357**,** 266-281.

HOLMAN, R. T., JOHNSON, S. B. & HATCH, T. F. (1982) A Case of Human Linolenic Acid Deficiency Involving Neurological Abnormalities. *American Journal of Clinical Nutrition,* 35**,** 617-623.

HOLMAN, R. T., JOHNSON, S. B. & OGBURN, P. L. (1991) Deficiency of Essential Fatty-Acids and Membrane Fluidity During Pregnancy and Lactation. *Proceedings of the National Academy of Sciences of the United States of America,* 88**,** 4835-4839.

HOOPER, L., THOMPSON, R. L., HARRISON, R. A., SUMMERBELL, C. D., NESS, A. R., MOORE, H., WORTHINGTON, H. V., DURRINGTON, P. N., HIGGINS, J. P. T., CAPPS, N. E., RIEMERSMA, R. A., EBRAHIM, S. B. & SMITH, G. D. (2006) Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *British Medical Journal,* 332**,** 752-755.

HORROCKS, L. A. & FAROOQUI, A. A. (2004) Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 70**,** 361-372.

HORWOOD, L. J. & FERGUSSON, D. M. (1998) Breastfeeding and later cognitive and academic outcomes. *Pediatrics,* 101**,** 8.

HOSHI, Y. (2007) Functional near-infrared spectroscopy: Current status and future prospects. *Journal of Biomedical Optics,* 12.

HOSHI, Y., KOBAYASHI, N. & TAMURA, M. (2001) Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. *Journal of Applied Physiology,* 90**,** 1657-1662.

HOSHI, Y. & TAMURA, M. (1993) Dynamic Multichannel near-Infrared Optical Imaging of Human Brain Activity. *Journal of Applied Physiology,* 75**,** 1842-1846.

HUANG, T. L., ZANDI, P. P., TUCKER, K. L., FITZPATRICK, A. L., KULLER, L. H., FRIED, L. P., BURKE, G. L. & CARLSON, M. C. (2005) Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon 4. *Neurology,* 65**,** 1409-1414.

HUPPERT, T. J., HOGE, R. D., DIAMOND, S. G., FRANCESCHINI, M. A. & BOAS, D. A. (2006) A temporal comparison of BOLD, ASL, and NIRS hemodynamic responses to motor stimuli in adult humans. *Neuroimage,* 29**,** 368-382.

INGWERSEN, J., DEFEYTER, M. A., KENNEDY, D. O., WESNES, K. A. & SCHOLEY, A. B. (2007) A low glycaemic index breakfast cereal preferentially prevents children's cognitive performance from declining throughout the morning. *Appetite,* 49**,** 240-244.

INNIS, S. M. (1991) Essential Fatty-Acids in Growth and Development. *Progress in Lipid Research,* 30**,** 39-103.

INNIS, S. M. (1993) Essential fatty acid requirements in human nutrition. *Can J Physiol Pharmacol,* 71**,** 699-706.

INNIS, S. M. & HANSEN, J. W. (1996) Plasma fatty acid responses, metabolic effects, and safety of microalgal and fungal oils rich in arachidonic and docosahexaenoic acids in healthy adults. *American Journal of Clinical Nutrition,* 64**,** 159-167.

ISSFAL (2004) *ISSFAL Newsletter,* 11**,** 12-16. Retrieved on March 10, 2010 from http://www.issfal.org.uk/images/stories/pdfs/Issnews022004.pdf.

ITOMURA, M., HAMAZAKI, K., SAWAZAKI, S., KOBAYASHI, M., TERASAWA, K., WATANABE, S. & HAMAZAKI, T. (2005) The effect of fish oil on physical aggression in schoolchildren - a randomized, double-blind, placebo-controlled trial. *Journal of Nutritional Biochemistry,* 16**,** 163-171.

JASDZEWSKI, G., STRANGMAN, G., WAGNER, J., KWONG, K. K., POLDRACK, R. A. & BOAS, D. A. (2003) Differences in the hemodynamic response to event-related motor and visual paradigms as measured by near-infrared spectroscopy. *Neuroimage,* 20**,** 479-488.

JENG, J.-Y., LEE, W.-H., TSAI, Y.-H., CHEN, C.-Y., CHAO, S.-Y. & HSIEH, R.-H. (2009) Functional Modulation of Mitochondria by Eicosapentaenoic Acid Provides Protection against Ceramide Toxicity to C6 Glioma Cells. *Journal of Agricultural and Food Chemistry,* 57**,** 11455-11462.

JENSEN, M. M., SKARSFELDT, T. & HOY, C. E. (1996) Correlation between level of (n - 3) polyunsaturated fatty acids in brain phospholipids and learning ability in rats. A multiple generation study. *Biochim Biophys Acta,* 1300**,** 203-9.

JOY, C. B., MUMBY-CROFT, R. & JOY, L. A. (2006) Polyunsaturated fatty acid supplementation for schizophrenia. *Cochrane Database of Systematic Reviews*.

KALMIJN, S. (2000) Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. *J Nutr Health Aging,* 4**,** 202-7.

KALMIJN, S., FESKENS, E. J., LAUNER, L. J. & KROMHOUT, D. (1997a) Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. *Am J Epidemiol,* 145**,** 33-41.

KALMIJN, S., LAUNER, L. J., OTT, A., WITTEMAN, J. C., HOFMAN, A. & BRETELER, M. M. (1997b) Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol,* 42**,** 776-82.

KALMIJN, S., VAN BOXTEL, M. P., OCKE, M., VERSCHUREN, W. M., KROMHOUT, D. & LAUNER, L. J. (2004a) Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology,* 62**,** 275-80.

KALMIJN, S., VAN BOXTEL, M. P. J., OCKE, M., VERSCHUREN, W. M. M., KROMHOUT, D. & LAUNER, L. J. (2004b) Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology,* 62**,** 275-280.

KANAMARU, Y., KIKUKAWA, A., MIYAMOTO, Y. & HIRAFUJI, M. (2008) Dimenhydrinate effect on cerebral oxygen status and salivary chromogranin-A during cognitive tasks. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* 32**,** 107-115.

KANG, J. X. & LEAF, A. (1996) Evidence that free polyunsaturated fatty acids modify Na+ channels by directly binding to the channel proteins. *Proceedings of the National Academy of Sciences of the United States of America,* 93**,** 3542-3546.

KATAN, M. B., DESLYPERE, J. P., VANBIRGELEN, A., PENDERS, M. & ZEGWAARD, M. (1997) Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *Journal of Lipid Research,* 38**,** 2012-2022.

KECK, P. E., MINTZ, J., MCELROY, S. L., FREEMAN, M. P., FRYE, T., ALTSHULER, L. L., KUPKA, R., NOLEN, W. A., LEVERICH, G. S., DENICOFF, K. D., GRUNZE, H., DUAN, N. H. & POST, R. M. (2006) Double-blind, randomized, placebo-controlled trials of ethyl-eicosapentanoate in the treatment of bipolar depression and rapid cycling bipolar disorder. *Biological Psychiatry,* 60**,** 1020-1022.

KENNEDY, D. O., PACE, S., HASKELL, C., OKELLO, E. J., MILNE, A. & SCHOLEY, A. B. (2006) Effects of cholinesterase inhibiting sage (Salvia officinalis) on mood, anxiety and performance on a psychological stressor battery. *Neuropsychopharmacology,* 31**,** 845-852.

KENNEDY, D. O., VEASEY, R., WATSON, A., DODD, F., JONES, E., MAGGINI, S. & HASKELL, C. (In press-a) Effects of high-dose B vitamin complex with vitamin C and minerals on subjective mood and performance in healthy males *Psychopharmacology*.

KENNEDY, D. O., WIGHTMAN, E., REAY, J., LEITZ, G., OKELLO, E. J., WILDE, A. & HASKELL, C. (In press-b) Effects of resveratrol on cerebral blood flow parameters and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *Am J Clin Nutr*.

KENNEDY, D. O., WIGHTMAN, E. L., REAY, J. L., LIETZ, G., OKELLO, E. J., WILDE, A. & HASKELL, C. F. (2010) Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. *American Journal of Clinical Nutrition,* 91**,** 1590-1597.

KEPPEL, G. (1991) *Design and analysis,* Upper Saddle River, NJ, Prentice Hall.

KIDD, P. M. (2007) Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. *Altern Med Rev,* 12**,** 207-27.

KIM, H. Y., AKBAR, M., LAU, A. & EDSALL, L. (2000) Inhibition of neuronal apoptosis by docosahexaenoic acid (22 : 6n-3) - Role of phosphatidylserine in antiapoptotic effect. *Journal of Biological Chemistry,* 275**,** 35215-35223.

KITAJKA, K., PUSKAS, L. G., ZVARA, A., HACKLER, L., BARCELO-COBLIJN, G., YEO, Y. K. & FARKAS, T. (2002) The role of n-3 polyunsaturated fatty acids in brain: Modulation of rat brain gene expression by dietary n-3 fatty acids. *Proceedings of the National Academy of Sciences of the United States of America,* 99**,** 2619-2624.

KITAURA, H., UOZUMI, N., TOHMI, M., YAMAZAKI, M., SAKIMURA, K., KUDOH, M., SHIMIZU, T. & SHIBUKI, K. (2007) Roles of nitric oxide as a vasodilator in neurovascular coupling of mouse somatosensory cortex. *Neuroscience Research,* 59**,** 160-171.

KNAPP, M. & PRINCE, M. (2007) Dementia UK - A report into the prevalence and cost of dementia. London, The Alzheimer's Society

KODAS, E., GALINEAU, L., BODARD, S., VANCASSEL, S., GUILLOTEAU, D., BESNARD, J. C. & CHALON, S. (2004) Serotoninergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. *J Neurochem,* 89**,** 695-702.

KOHL, M., NOLTE, C., HEEKEREN, H. R., HORST, S., SCHOLZ, U., OBRIG, H. & VILLRINGER, A. (1998) Determination of the wavelength dependence of the differential pathlength factor from near-infrared pulse signals. *Physics in Medicine and Biology,* 43**,** 1771-1782.

KRAMER, M. S., ABOUD, F., MIRONOVA, E., VANILOVICH, I., PLATT, R. W., MATUSH, L., IGUMNOV, S., FOMBONNE, E., BOGDANOVICH, N., DUCRUET, T., COLLET, J. P., CHALMERS, B., HODNETT, E., DAVIDOVSLZY, S., SKUGAREVSKY, O., TROFIMOVICH, O., KOZLOVA, L. & SHAPIRO, S. (2008) Breastfeeding and child cognitive development - New evidence from a large randomized trial. *Archives of General Psychiatry,* 65**,** 578-584.

KROGER, E., VERREAULT, R., CARMICHAEL, P. H., LINDSAY, J., JULIEN, P., DEWAILLY, E., AYOTTE, P. & LAURIN, D. (2009) Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. *Am J Clin Nutr,* 90**,** 184-92.

KROMHOUT, D., FESKENS, E. J. M. & BOWLES, C. H. (1995) The Protective Effect of a Small Amount of Fish on Coronary Heart-Disease Mortality in an Elderly Population. *International Journal of Epidemiology,* 24**,** 340-345.

LANDFIELD, P. W., MCGAUGH, J. L. & LYNCH, G. (1978) Impaired Synaptic Potentiation Processes in Hippocampus of Aged, Memory-Deficient Rats. *Brain Research,* 150**,** 85-101.

LAWRENCE, N. S., ROSS, T. J. & STEIN, E. A. (2002) Cognitive mechanisms of nicotine on visual attention. *Neuron,* 36**,** 539-548.

LE MOAL, M. & SIMON, H. (1991) Mesocorticolimbic Dopaminergic Network Functional and Regulatory Roles. *Physiological Reviews,* 71**,** 155-234.

LEE, Y. H., KIM, H. J., RHO, Y. H., CHOI, S. J., JI, J. D. & SONG, G. G. (2004) Interleukin-1 receptor antagonist gene polymorphism and rheumatoid arthritis. *Rheumatology International,* 24**,** 133-136.

LEON, H., SHIBATA, M. C., SIVAKUMARAN, S., DORGAN, M., CHATTERLEY, T. & TSUYUKI, R. T. (2008) Effect of fish oil on arrhythmias and mortality: systematic review. *British Medical Journal,* 337**,** 8.

LEVANT, B., RADEL, J. D. & CARLSON, S. E. (2006) Reduced brain DHA content after a single reproductive cycle in female rats fed a diet deficient in N-3 polyunsaturated fatty acids. *Biological Psychiatry,* 60**,** 987-990.

LI, P., KIM, S. W., LI, X. L., DATTA, S., POND, W. G. & WU, G. Y. (2008) Dietary supplementation with cholesterol and docosahexaenoic acid increases the activity of the arginine-nitric oxide pathway in tissues of young pigs. *Nitric Oxide-Biology and Chemistry,* 19**,** 259-265.

LICHTENSTEIN, A. H., APPEL, L. J., BRANDS, M., CARNETHON, M., DANIELS, S., FRANCH, H. A., FRANKLIN, B., KRIS-ETHERTON, P., HARRIS, W. S., HOWARD, B., KARANJA, N., LEFEVRE, M., RUDEL, L., SACKS, F., VAN HORN, L., WINSTON, M. & WYLIE-ROSETT, J. (2006) Diet and lifestyle recommendations revision 2006 - A scientific statement from the American Heart Association Nutrition Committee (vol 114, pg 82, 2006). *Circulation,* 114**,** 82-+.

LIM, S. Y., HOSHIBA, J. & SALEM, N. (2005) An extraordinary degree of structural specificity is required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid substitution for docosahexaenoic acid leads to a loss in spatial task performance. *Journal of Neurochemistry,* 95**,** 848-857.

LIM, W. S., GAMMACK, J. K., VAN NIEKERK, J. & DANGOUR, A. D. (2006) Omega 3 fatty acid for the prevention of dementia. *Cochrane Database of Systematic Reviews*.

LOVIBOND, S. H. & LOVIBOND, P. F. (1995) *Manual for the Depression, Anxiety and Stress Scales (2nd Ed),* Sydney, Psychology Foundation.

LUCAS, A., MORLEY, R., COLE, T. J., LISTER, G. & LEESON-PAYNE, C. (1992) Breast milk and subsequent intelligence quotient in children born preterm. *The Lancet,* 339**,** 261-264.

LUCAS, A., STAFFORD, M., MORLEY, R., ABBOTT, R., STEPHENSON, T., MACFADYEN, U., ELIAS-JONES, A. & CLEMENTS, H. (1999) Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial. *The Lancet,* 354**,** 1948-1954.

MAES, M., SONG, C., LIN, A. H., DE JONGH, R., VAN GASTEL, A., KENIS, G., BOSMANS, E., DE MEESTER, I., BENOY, I., NEELS, H., DEMEDTS, P., JANCA, A., SCHARPE, S. & SMITH, R. S. (1998) The effects of psychological stress on humans: Increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine,* 10**,** 313-318.

MAKRIDES, M., CROWTHER, C. A., GIBSON, R. A., GIBSON, R. S. & SKEAFF, C. M. (2003) Docosahexaenoic acid and post-partum depression - is there a link? *Asia Pac J Clin Nutr,* 12 Suppl**,** S37.

MAKRIDES, M., NEUMANN, M., SIMMER, K., PATER, J. & GIBSON, R. (1995) Are long-chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet,* 345**,** 1463-8.

MAKRIDES, M., NEUMANN, M. A., BYARD, R. W., SIMMER, K. & GIBSON, R. A. (1994) Fatty-Acid Composition of Brain, Retina, and Erythrocytes in Breast-Fed and Formula-Fed Infants. *American Journal of Clinical Nutrition,* 60**,** 189-194.

MAKRIDES, M., NEUMANN, M. A., SIMMER, K. & GIBSON, R. A. (2000) A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: A randomized, controlled trial. *Pediatrics,* 105**,** 32-38.

MAMALAKIS, G., JANSEN, E., CREMERS, H., KIRIAKAKIS, M., TSIBINOS, G. & KAFATOS, A. (2006a) Depression and adipose and serum cholesteryl ester polyunsaturated fatty acids in the survivors of the seven countries study population of Crete. *Eur J Clin Nutr,* 60**,** 1016-23.

MAMALAKIS, G., KIRIAKAKIS, M., TSIBINOS, G., HATZIS, C., FLOURI, S., MANTZOROS, C. & KAFATOS, A. (2006b) Depression and serum adiponectin and adipose omega-3 and omega-6 fatty acids in adolescents. *Pharmacology Biochemistry and Behaviour,* 85**,** 474-479.

MAMALAKIS, G., KIRIAKAKIS, M., TSIBINOS, G. & KAFATOS, A. (2004a) Depression and adipose polyunsaturated fatty acids in an adolescent group. *Prostaglandins Leukot Essent Fatty Acids,* 71**,** 289-94.

MAMALAKIS, G., KIRIAKAKIS, M., TSIBINOS, G. & KAFATOS, A. (2004b) Depression and adipose polyunsaturated fatty acids in the survivors of the

Seven Countries Study population of Crete. *Prostaglandins Leukot Essent Fatty Acids,* 70**,** 495-501.

MAMALAKIS, G., TORNARITIS, M. & KAFATOS, A. (2002) Depression and adipose essential polyunsaturated fatty acids. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 67**,** 311-318.

MARANGELL, L. B., MARTINEZ, J. M., ZBOYAN, H. A., CHONG, H. & PURYEAR, L. J. (2004) Omega-3 fatty acids for the prevention of postpartum depression: Negative data from a preliminary, open-label pilot study. *Depression and Anxiety,* 19**,** 20-23.

MARANGELL, L. B., MARTINEZ, J. M., ZBOYAN, H. A., SEUNG KIM, H. F. & PUYEAR, L. J. (2003) A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. *American Journal of Psychiatry,* 160**,** 996-998.

MARIANI, E., MONASTERO, R. & MECOCCI, P. (2007) Mild cognitive impairment: a systematic review. *J Alzheimers Dis,* 12**,** 23-35.

MARKESBERY, W. R. (1997) Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine,* 23**,** 134-147.

MARKUS, C. R., FIRK, C., GERHARDT, C., KLOEK, J. & SMOLDERS, G. F. (2008) Effect of different tryptophan sources on amino acids availability to the brain and mood in healthy volunteers. *Psychopharmacology,* 201**,** 107-114.

MARTEK (2006) Memory Improvement With Docosahexaenoic Acid Study (MIDAS).

MARX, E., DEUTSCHLANDER, A., STEPHAN, T., DIETERICH, M., WIESMANN, M. & BRANDT, T. (2004) Eyes open and eyes closed as rest conditions: impact on brain activation patterns. *Neuroimage,* 21**,** 1818-1824.

MASOOD, A., STARK, K. D. & SALEM, N. (2005) A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. *J Lipid Res,* 46**,** 2299-2305.

MASTERSON, J., STUART, M., DIXON, M. & LOVEJOY, S. (2003) Children's Printed Word Database: Economic and Social Research Council funded project, R00023406.

MATSUMOTO, T., NAKAYAMA, N., ISHIDA, K., KOBAYASHI, T. & KAMATA, K. (2009) Eicosapentaenoic Acid Improves Imbalance between Vasodilator and Vasoconstrictor Actions of Endothelium-Derived Factors in Mesenteric Arteries from Rats at Chronic Stage of Type 2 Diabetes. *Journal of Pharmacology and Experimental Therapeutics,* 329**,** 324-334.

MCGAHON, B. M., MARTIN, D. S., HORROBIN, D. F. & LYNCH, M. A. (1999) Age-related changes in synaptic function: analysis of the effect of dietary supplementation with omega-3 fatty acids. *Neuroscience,* 94**,** 305-14.

MCKEITH, I. & CUMMINGS, J. (2005) Behavioural changes and psychological symptoms in dementia disorders. *Lancet Neurology,* 4**,** 735-742.

MCNAMARA, R. K. & CARLSON, S. E. (2006) Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids,* 75**,** 329-49.

MCNAMARA, R. K., HAHN, C. G., JANDACEK, R., RIDER, T., TSO, P., STANFORD, K. E. & RICHTAND, N. M. (2007) Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biological Psychiatry,* 62**,** 17-24.

MELLOR, H. & PARKER, P. J. (1998) The extended protein kinase C superfamily. *Biochemical Journal,* 332**,** 281-292.

MENON, N. K. & DHOPESHWARKAR, G. A. (1983) Essential Fatty-Acid Deficiency and Brain-Development. *Progress in Lipid Research,* 21**,** 309-326.

METHEREL, A. H., ARMSTRONG, J. M., PATTERSON, A. C. & STARK, K. D. (2009) Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. *Prostaglandins Leukot Essent Fatty Acids,* 81**,** 23-9.

MILES, E. A., ALLEN, E. & CALDER, P. C. (2002) In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. *Cytokine,* 20**,** 215-223.

MINAMI, M., KIMURA, S., ENDO, T., HAMAUE, N., HIRAFUJI, M., TOGASHI, H., MATSUMOTO, M., YOSHIOKA, M., SAITO, H., WATANABE, S., KOBAYASHI, T. & OKUYAMA, H. (1997) Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. *Pharmacology Biochemistry and Behavior,* 58**,** 1123-1129.

MOORE, S. A. (2001) Polyunsaturated fatty acid synthesis and release by brain-derived cells in vitro. *Journal of Molecular Neuroscience,* 16**,** 195-200.

MORI, T. A., BEILIN, L. J., BURKE, V., MORRIS, J. & RITCHIE, J. (1997) Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. *Arteriosclerosis Thrombosis and Vascular Biology,* 17**,** 279-286.

MORI, T. A., PUDDEY, I. B., BURKE, V., CROFT, K. D., DUNSTAN, D. W., RIVERA, J. H. & BEILIN, L. J. (2000) Effect of omega 3 fatty acids on oxidative stress in humans: GC-MS measurement of urinary F-2-isoprostane excretion. *Redox Report,* 5**,** 45-46.

MORI, T. A. & WOODMAN, R. J. (2006) The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Current Opinion in Clinical Nutrition and Metabolic Care,* 9**,** 95-104.

MORIGUCHI, T., GREINER, R. S. & SALEM, N. (2000) Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. *Journal of Neurochemistry,* 75**,** 2563-2573.

MORIGUCHI, T., LOEWKE, J., GARRISON, M., CATALAN, J. N. & SALEM, N., JR. (2001) Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum. *J Lipid Res,* 42**,** 419-27.

MORIGUCHI, T. & SALEM, N., JR. (2003) Recovery of brain docosahexaenoate leads to recovery of spatial task performance. *J Neurochem,* 87**,** 297-309.

MORRIS, M. C., EVANS, D. A., BIENIAS, J. L., TANGNEY, C. C., BENNETT, D. A., WILSON, R. S., AGGARWAL, N. & SCHNEIDER, J. (2003) Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Arch Neurol,* 60**,** 940-6.

MORRIS, M. C., EVANS, D. A., BIENIAS, J. L., TANGNEY, C. C. & WILSON, R. S. (2002) Vitamin E and cognitive decline in older persons. *Archives of Neurology,* 59**,** 1125-1132.

MORRIS, M. C., EVANS, D. A., TANGNEY, C. C., BIENIAS, J. L. & WILSON, R. S. (2005) Fish consumption and cognitive decline with age in a large community study. *Arch Neurol,* 62**,** 1849-53.

MORRIS, M. C., SACKS, F. & ROSNER, B. (1993) Does Fish-Oil Lower Blood-Pressure - a Metaanalysis of Controlled Trials. *Circulation,* 88**,** 523-533.

MORRIS, R. (1984) Developments of a Water-Maze Procedure for Studying Spatial-Learning in the Rat. *Journal of Neuroscience Methods,* 11**,** 47-60.

MORSE, N. L. (2009) A meta-analysis of blood fatty acids in people with learning disorders with particular interest in arachidonic acid. *Prostaglandins Leukot Essent Fatty Acids,* 81**,** 373-89.

MRAK, R. E. & GRIFFIN, W. S. T. (2001) Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiology of Aging,* 22**,** 903-908.

NARAYANAN, B. A., NARAYANAN, N. K. & REDDY, B. S. (2001) Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells. *International Journal of Oncology,* 19**,** 1255-1262.

NESS, A. R., GALLACHER, J. E. J., BENNETT, P. D., GUNNELL, D. J., ROGERS, P. J., KESSLER, D. & BURR, M. L. (2003) Advice to eat fish and mood: A randomised controlled trial in men with angina. *Nutritional Neuroscience,* 6**,** 63-65.

NESTEL, P. J. (2000) Fish oil and cardiovascular disease: lipids and arterial function. *American Journal of Clinical Nutrition,* 71**,** 228S-231S.

NEURINGER, M. & CONNOR, W. E. (1986) n-3 fatty acids in the brain and retina: evidence for their essentiality. *Nutr Rev,* 44**,** 285-94.

NG, K. H., MEYER, B. J., REECE, L. & SINN, N. (2009) Dietary PUFA intakes in children with attention-deficit/hyperactivity disorder symptoms. *British Journal of Nutrition,* 102**,** 1635-1641.

NIEOULLON, A. (2002) Dopamine and the regulation of cognition and attention. *Progress in Neurobiology,* 67**,** 53-83.

NOAGHIUL, S. & HIBBELN, J. R. (2003) Cross-national comparisons of seafood consumption and rates of bipolar disorders. *Am J Psychiatry,* 160**,** 2222-7.

OAKESHOTT, I. (2006) Plan to give pupils fish oil capsules. The Sunday Times. Retrieved on June 12, 2006, from http://www.timesonline.co.uk/tol/news/uk/article673739.ece.

OATTS, J. (2006) Watchdog cracks down on St Ivel milk ad claims. (June 19, 2006). Retrieved January 15, 2010. http://www.brandrepublic.com/news/565082/watchdog-cracks-down-st-ivel-milk-ad-claims/.

OBRIG, H. & VILLRINGER, A. (2003) Beyond the visible - Imaging the human brain with light. *Journal of Cerebral Blood Flow and Metabolism,* 23**,** 1-18.

OHARA, K. (2007) The n-3 polyunsaturated fatty acid/dopamine hypothesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*.

ONS (2009) Ageing: Fastest increase in the 'oldest old' Office for National Statistics.

ORDOVAS, J. M., CORELLA, D., CUPPLES, L. A., DEMISSIE, S., KELLEHER, A., COLTELL, O., WILSON, P. W. F., SCHAEFER, E. J. & TUCKER, K. (2002) Polyunsaturated fatty acids modulate the effects of the APOA1 G-A polymorphism on HDL-cholesterol concentrations in a sex-specific manner: the Framingham Study. *American Journal of Clinical Nutrition,* 75**,** 38-46.

OSENDARP, S. J. M., BAGHURST, K. I., BRYAN, J., CALVARESI, E., HUGHES, D., HUSSAINI, M., KARYADI, S. J. M., VAN KLINKEN, B. J.-W., VAN DER KNAAP, H. C. M., LUKITO, W., MIKARSA, W., TRANSLER, C. & WILSON, C. (2007) Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia. *Am J Clin Nutr,* 86**,** 1082-93.

OSHER, Y., BERSUDSKY, Y. & BELMAKER, R. H. (2005) Omega-3 eicosapentaenoic acid in bipolar depression: report of a small open-label study. *J Clin Psychiatry,* 66**,** 726-9.

OWEN, A. M., EVANS, A. C. & PETRIDES, M. (1996) Evidence for a two-stage model of spatial working memory processing within the lateral frontal cortex: A positron emission tomography study. *Cerebral Cortex,* 6**,** 31-38.

PALEOLOGOS, M., CUMMING, R. G. & LAZARUS, R. (1998) Cohort study of vitamin C intake and cognitive impairment. *American Journal of Epidemiology,* 148**,** 45-50.

PANERAI, R. B. (2009) Transcranial Doppler for evaluation of cerebral autoregulation. *Clinical Autonomic Research,* 19**,** 197-211.

PARY, R., LEWIS, S., MATUSCHKA, P. R. & LIPPMANN, S. (2002) Attention-deficit/hyperactivity disorder: An update. *Southern Medical Journal,* 95**,** 743-749.

PATARCA, R. (2001) Cytokines and chronic fatigue syndrome. IN SORG, B. A. & BELL, I. R. (Eds.) *Role of Neural Plasticity in Chemical Intolerance.* New York, New York Acad Sciences.

PEET, M. (2003) Eicosapentaenoic acid in the treatment of schizophrenia and depression: rationale and preliminary double-blind clinical trial results. *Prostaglandins Leukot Essent Fatty Acids,* 69**,** 477-85.

PEET, M., BRIND, J., RAMCHAND, C. N., SHAH, S. & VANKAR, G. K. (2001) Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia. *Schizophrenia Research,* 49**,** 243-251.

PEET, M. & HORROBIN, D. F. (2002a) A dose-ranging exploratory study of the effects of ethyl-eicosapentaenoate in patients with persistent schizophrenic symptoms. *Journal of Psychiatric Research,* 36**,** 7-18.

PEET, M. & HORROBIN, D. F. (2002b) A dose-ranging study of the, effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Archives of General Psychiatry,* 59**,** 913-919.

PEET, M., LAUGHARNE, J. D. E., MELLOR, J. & RAMCHAND, C. N. (1995) Essential fatty acid deficiency In erythrocyte membranes from chronic schizophrenic patients, and the clinical effects of dietary supplementation. *2nd International Workshop on Cell Membrane Pathology in Schizophrenia.* London, England, Churchill Livingstone.

PEET, M. & MELLOR, J. (1998) Double-blind placebo controlled trial of N-3 polyunsaturated fatty acids as an adjunct to neuroleptics. *Schizophrenia Research,* 29**,** 160-161.

PEET, M., MURPHY, B., SHAY, J. & HORROBIN, D. (1998a) Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry,* 43**,** 315-9.

PEET, M., MURPHY, B., SHAY, J. & HORROBIN, D. (1998b) Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biological Psychiatry,* 43**,** 315-319.

PETERS, R., PETERS, J., WARNER, J., BECKETT, N. & BULPITT, C. (2008) Alcohol, dementia and cognitive decline in the elderly: a systematic review. *Age and Ageing,* 37**,** 505-512.

POLLITT, E. (1993) Iron-Deficiency and Cognitive Function. *Annual Review of Nutrition,* 13**,** 521-537.

PRATICO, D. & TROJANOWSKI, J. Q. (2000) Inflammatory hypotheses: novel mechanisms of Alzheimer's neurodegeneration and new therapeutic targets? Commentary. *Neurobiology of Aging,* 21**,** 441-445.

QUINN, J. (2007) A Randomized Double-Blind Placebo-Controlled Trial Of The Effects Of Docosahexaenoic Acid (DHA) In Slowing The Progression Of Alzheimer's Disease.

RANJEKAR, P. K., HINGE, A., HEGDE, M. V., GHATE, M., KALE, A., SITASAWAD, S., WAGH, U. V., DEBSIKDAR, V. B. & MAHADIK, S. P. (2003) Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. *Psychiatry Research,* 121**,** 109-122.

REAY, J. L., KENNEDY, D. O. & SCHOLEY, A. B. (2006) Effects of Panax ginseng, consumed with and without glucose, on blood glucose levels and cognitive performance during sustained 'mentally demanding' tasks. *Journal of Psychopharmacology,* 20**,** 771-781.

REYNOLDS, E. H. (2002) Folic acid, ageing, depression, and dementia. *British Medical Journal,* 324**,** 1512-1515.

RICHARDSON, A. J. (2006) Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *International Review of Psychiatry,* 18**,** 155-172.

RICHARDSON, A. J. (2008) n-3 Fatty acids and mood: the devil is in the detail. *Br J Nutr,* 99**,** 221-3.

RICHARDSON, A. J., CALVIN, C. M., CLISBY, C., SCHOENHEIMER, D. R., MONTGOMERY, P., HALL, J. A., HEBB, G., WESTWOOD, E., TALCOTT, J. B. & STEIN, J. F. (2000) Fatty acid deficiency signs predict the severity of reading and related difficulties in dyslexic children. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 63**,** 69-74.

RICHARDSON, A. J. & MONTGOMERY, P. (2005) The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics,* 115**,** 1360-6.

RICHARDSON, A. J. & PURI, B. K. (2002) A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties. *Progress in Neuro-Psychopharmacology % Biological Psychiatry,* 26**,** 233-239.

RICHARDSON, A. J. & ROSS, M. A. (2000) Fatty acid metabolism in neurodevelopmental disorder: a new perspective on associations between attention-deficit hyperactivity disoder, dyslexia, dyspraxia and the autistic spectrum. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 63**,** 1-9.

RICHTER, M. M., ZIERHUT, K. C., DRESLER, T., PLICHTA, M. M., EHLIS, A. C., REISS, K., PEKRUN, R. & FALLGATTER, A. J. (2009) Changes in cortical blood oxygenation during arithmetical tasks measured by near-infrared spectroscopy. *Journal of Neural Transmission,* 116**,** 267-273.

RISSANEN, T., VOUTILAINEN, S., NYYSSONEN, K., LAKKA, T. A. & SALONEN, J. T. (2000) Fish oil-derived fatty acids, docosahexaenoic acid and

docosapentaenoic acid, and the risk of acute coronary events - The kuopio ischaemic heart disease risk factor study. *Circulation,* 102**,** 2677-2679.

ROGERS, P. J., APPLETON, K. M., KESSLER, D., PETERS, T. J., GUNNELL, D., HAYWARD, R. C., HEATHERLEY, S. V., CHRISTIAN, L. M., MCNAUGHTON, S. A. & NESS, A. R. (2008) No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. *British Journal of Nutrition,* 99**,** 421-431.

ROGERS, P. J., MARTIN, J., SMITH, C., HEATHERLEY, S. V. & SMIT, H. J. (2003) Absence of reinforcing, mood and psychomotor performance effects of caffeine in habitual non-consumers of caffeine. *Psychopharmacology,* 167**,** 54-62.

ROSENTHAL, J. M., AMIEL, S. A., YAGUEZ, L., BULLMORE, E., HOPKINS, D., EVANS, M., PERNET, A., REID, H., GIAMPIETRO, V., ANDREW, C. M., SUCKLING, J., SIMMONS, A. & WILLIAMS, S. C. R. (2001) The effect of acute hypoglycemia on brain function and activation - A functional magnetic resonance imaging study. *Diabetes,* 50**,** 1618-1626.

RYAN, A. S. & NELSON, E. B. (2008) Assessing the Effect of Docosahexaenoic Acid on Cognitive Functions in Healthy, Preschool Children: A Randomized, Placebo-Controlled, Double-Blind Study. *Clin Pediatr (Phila)*.

SACN/COT (2004) *Advice on Fish Consumption:Benefits and Risks.,* London, TSO.

SAGDUYU, K., DOKUCU, M. E., EDDY, B. A., CRAIGEN, G., BALDASSANO, C. F. & YILDIZ, A. (2005) Omega-3 fatty acids decreased irritability of patients with bipolar disorder in an add-on, open label study. *Nutr J,* 4**,** 6.

SALVATI, S., NATALI, F., ATTORRI, L., DI BENEDETTO, R., LEONARDI, F., DI BIASE, A., FERRI, F., FORTUNA, S., LORENZINI, P., SANCHEZ, M., RICCERI, L. & VITELLI, L. (2008) Eicosapentaenonic add stimulates the expression of myelin proteins in rat brain. *Journal of Neuroscience Research,* 86**,** 776-784.

SANDERS, T. & EMERY, P. (2003) *Molecular Basis of Human Nutrition* London, Taylor & Francis.

SANDERS, T. A. (2009) DHA status of vegetarians. *Prostaglandins Leukot Essent Fatty Acids,* 81**,** 137-141.

SANDERS, T. A. B. & REDDY, S. (1994) Vegetarian Diets and Children. *American Journal of Clinical Nutrition,* 59**,** S1176-S1181.

SCHAEFER, E. J., BONGARD, V., BEISER, A. S., LAMON-FAVA, S., ROBINS, S. J., AU, R., TUCKER, K. L., KYLE, D. J., WILSON, P. W. F. & WOLF, P. A. (2006) Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease - The Framingham heart study. *Archives of Neurology,* 63**,** 1545-1550.

SCHECKLMANN, M., EHLIS, A. C., PLICHTA, M. M. & FALLGATTER, A. J. (2008) Functional near-infrared spectroscopy: A long-term reliable tool for measuring brain activity during verbal fluency. *Neuroimage,* 43**,** 147-155.

SCHINS, A., CRIJNS, H. J., BRUMMER, R. J., WICHERS, M., LOUSBERG, R., CELIS, S. & HONIG, A. (2007) Altered omega-3 polyunsaturated fatty acid status in depressed post-myocardial infarction patients. *Acta Psychiatr Scand,* 115**,** 35-40.

SCHLEBUSCH, L., BOSCH, B. A., POLGLASE, G., KLEINSCHMIDT, I., PILLAY, B. J. & CASSIMJEE, M. H. (2000) A double-blind, placebo-controlled, double-centre study of the effects of an oral multivitamin-mineral combination on stress. *South African Medical Journal,* 90**,** 1216-1223.

SCHLUTER, N. D., KRAMS, M., RUSHWORTH, M. F. S. & PASSINGHAM, R. E. (2001) Cerebral dominance for action in the human brain: the selection of actions. *Neuropsychologia,* 39**,** 105-113.

SCHMITZ, G. & ECKER, J. (2008) The opposing effects of n-3 and n-6 fatty acids. *Prog Lipid Res,* 47**,** 147-55.

SCHOLEY, A. B. & KENNEDY, D. O. (2004) Cognitive and physiological effects of an "energy drink": an evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions. *Psychopharmacology,* 176**,** 320-330.

SCHOLEY, A. B., MOSS, M. C., NEAVE, N. & WESNES, K. (1999) Cognitive performance, hyperoxia, and heart rate following oxygen administration in healthy young adults. *Physiology & Behavior,* 67**,** 783-789.

SCHUCHARDT, J. P., HUSS, M., STAUSS-GRABO, M. & HAHN, A. (2009) Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur J Pediatr*.

SEEBUNGKERT, B. & LYNCH, J. W. (2002) Effects of polyunsaturated fatty acids on voltage-gated K+ and Na+ channels in rat olfactory receptor neurons. *European Journal of Neuroscience,* 16**,** 2085-2094.

SEGERSTROM, S. C. & MILLER, G. E. (2004) Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin,* 130**,** 601-630.

SELEK, S., SAVAS, H. A., GERGERLIOGLU, H. S., BULUT, M. & YILMAZ, H. R. (2008) Oxidative imbalance in adult attention deficit/hyperactivity disorder. *Biological Psychology,* 79**,** 256-259.

SHAH, A. P., ICHIUJI, A. M., HAN, J. K., TRAINA, M., EL-BIALY, A., MEYMANDI, S. K. & WACHSNER, R. Y. (2007) Cardiovascular and endothelial effects of fish oil supplementation in healthy volunteers. *J Cardiovasc Pharmacol Ther,* 12**,** 213-9.

SHALLICE, T. (1982) SPECIFIC IMPAIRMENTS OF PLANNING. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 298**,** 199-209.

SHUKITT-HALE, B., MOUZAKIS, G. & JOSEPH, J. A. (1998) Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Experimental Gerontology,* 33**,** 615-624.

SILVERS, K. M. & SCOTT, K. M. (2002) Fish consumption and self-reported physical and mental health status. *Public Health Nutr,* 5**,** 427-31.

SILVERS, K. M., WOOLLEY, C. C., HAMILTON, F. C., WATTS, P. M. & WATSON, R. A. (2005) Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. *Prostaglandins Leukot Essent Fatty Acids,* 72**,** 211-8.

SIMOPOULOS, A. P. (1991) Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr,* 54**,** 438-63.

SIMOPOULOS, A. P. (2000) Human requirement for N-3 polyunsaturated fatty acids. *Poult Sci,* 79**,** 961-70.

SIMOPOULOS, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy,* 56**,** 365-379.

SIMOPOULOS, A. P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine,* 233**,** 674-688.

SLATER, S. J., KELLY, M., YEAGER, M. D., LARKIN, J., HO, C. J. & STUBBS, C. D. (1995) Polyunsaturation in cell membranes and lipid bilayers and its effects on membrane proteins. *2nd Congress of the International-Society-for-the-Study-of-Fatty-Acids-and-Lipids.* Bethesda, Md, Amer Oil Chemists Soc.

SLIWINSKI, S., CROONENBERGHS, J., CHRISTOPHE, A., DEBOUTTE, D. & MAES, M. (2006) Polyunsaturated fatty acids: Do they have a role in the pathophysiology of autism? *Neuroendocrinology Letters,* 27**,** 465-471.

SNODGRASS, J. G. & VANDERWART, M. (1980) Standardized Set of 260 Pictures - Norms for Name Agreement, Image Agreement, Familiarity, and Visual Complexity. *Journal of Experimental Psychology-Human Learning and Memory,* 6**,** 174-215.

SONGUR, A., SARSILMAZ, M., SOGUT, S., OZYURT, B., OZYURT, H., ZARARSIZ, I. & TURKOGLU, A. O. (2004) Hypothalamic superoxide dismutase, xanthine oxidase, nitric oxide, and malondialdehyde in rats fed with fish omega-3 fatty acids. *Progress in Neuro-Psychopharmacology & Biological Psychiatry,* 28**,** 693-698.

STEINBRINK, J., VILLRINGER, A., KEMPF, F., HAUX, D., BODEN, S. & OBRIG, H. (2005) Illuminating the BOLD signal: combined fMRI-fNIRS studies. *International School on Magnetic Resonance and Brain Function.* Erice, ITALY.

STEVENS, L., ZHANG, W., PECK, L., KUCZEK, T., GREVSTAD, N., MAHON, A., ZENTALL, S. S., ARNOLD, I. E. & BURGESS, J. R. (2003) EFA

supplementation in children with inattention, hyperactivity, and other disruptive behaviors. *Lipids,* 38**,** 1007-1021.

STEVENS, L. J., ZENTALL, S. S., ABATE, M. L., KUCZEK, T. & BURGESS, J. R. (1996) Omega-3 fatty acids in boys with behavior, learning, and health problems. *Physiol Behav,* 59**,** 915-20.

STEVENS, L. J., ZENTALL, S. S., DECK, J. L., ABATE, M. L., WATKINS, B. A., LIPP, S. R. & BURGESS, J. R. (1995) Essential Fatty-Acid Metabolism in Boys with Attention-Deficit Hyperactivity Disorder. *American Journal of Clinical Nutrition,* 62**,** 761-768.

STILLWELL, W. & WASSALL, S. R. (2003) Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids,* 126**,** 1-27.

STOLL, A. L., SEVERUS, W. E., FREEMAN, M. P., RUETER, S., ZBOYAN, H. A., DIAMOND, E., CRESS, K. K. & MARANGELL, L. B. (1999) Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch Gen Psychiatry,* 56**,** 407-12.

STORDY, B. J. (2000) Dark adaptation, motor skills, docosahexaenoic acid, and dyslexia. *American Journal of Clinical Nutrition,* 71**,** 323S-326S.

STROOP, J. R. (1992) Studies of Interference in Serial Verbal Reactions (Reprinted from Journal Experimental-Psychology, Vol 18, Pg 643-662, 1935). *J Exp Psychol Gen,* 121**,** 15-23.

SU, K. P., HUANG, S. Y., CHIU, C. C. & SHEN, W. W. (2003) Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur Neuropsychopharmacol,* 13**,** 267-71.

SUBLETTE, M. E., MILAK, M. S., HIBBELN, J. R., FREED, P. J., OQUENDO, M. A., MALONE, K. M., PARSEY, R. V. & MANN, J. J. (2009) Plasma polyunsaturated fatty acids and regional cerebral glucose metabolism in major depression. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 80**,** 57-64.

SUN, M.-K. & ALKON, D. L. (2006) Protein kinase C pharmacology: perspectives on therapeutic potentials as antidementic and cognitive agents. *Recent Pat CNS Drug Discov,* 1**,** 147-56.

SURETTE, M. E. (2008) The science behind dietary omega-3 fatty acids. *Canadian Medical Association Journal,* 178**,** 177-180.

SWAN, G. E. & LESSOV-SCHLAGGAR, C. N. (2007) The effects of tobacco smoke and nicotine on cognition and the brain. *Neuropsychology Review,* 17**,** 259-273.

TAMURA, M., HOSHI, Y. & OKADA, F. (1997) Localized near-infrared spectroscopy and functional optical imaging of brain activity. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 352**,** 737-742.

TANSKANEN, A., HIBBELN, J. R., TUOMILEHTO, J., UUTELA, A., HAUKKALA, A., VIINAMAKI, H., LEHTONEN, J. & VARTIAINEN, E. (2001) Fish consumption

and depressive symptoms in the general population in Finland. *Psychiatr Serv,* 52**,** 529-31.

TASSONI, D., KAUR, G., WEISINGER, R. S. & SINCLAIR, A. J. (2007) The role of eicosanoids in the brain. *10th Asian Congress of Nutrition.* Taipei, TAIWAN, H E C Press, Healthy Eating Club Pty Ltd.

TASSONI, D., KAUR, G., WEISINGER, R. S. & SINCLAIR, A. J. (2008) The role of eicosanoids in the brain. *Asia Pac J Clin Nutr,* 17 Suppl 1**,** 220-8.

TAYLOR, K. E., HIGGINS, C. J., CALVIN, C. M., HALL, J. A., EASTON, T., MCDAID, A. M. & RICHARDSON, A. J. (2000) Dyslexia in adults is associated with clinical signs of fatty acid deficiency. *Prostaglandins Leukotrienes and Essential Fatty Acids,* 63**,** 75-78.

TERANO, T., FUJISHIRO, S., BAN, T., YAMAMOTO, K., TANAKA, T., NOGUCHI, Y., TAMURA, Y., YAZAWA, K. & HIRAYAMA, T. (1998) Docosahexaenoic acid supplementation improves the moderately severe dementia from thrombotic cerebrovascular diseases. *3rd Congress of the International-Society-for-the-Study-of-Fatty-Acids-and-Lipids.* Lyon, France, Amer Oil Chemists Soc a O C S Press.

THEMEDICA (2009) The UK Dietary Supplements Industry Overview.

TIEMEIER, H., VAN TUIJL, H. R., HOFMAN, A., KILIAAN, A. J. & BRETELER, M. M. (2003) Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam Study. *Am J Clin Nutr,* 78**,** 40-6.

TOICHI, M., FINDLING, R. L., KUBOTA, Y., CALABRESE, J. R., WIZNITZER, M., MCNAMARA, N. K. & YAMAMOTO, K. (2004) Hemodynamic differences in the activation of the prefrontal cortex: attention vs. higher cognitive processing. *Neuropsychologia,* 42**,** 698-706.

TOONE, B. K., OKOCHA, C. I., SIVAKUMAR, K. & SYED, G. M. (2000) Changes in regional cerebral blood flow due to cognitive activation among patients with schizophrenia. *British Journal of Psychiatry,* 177**,** 222-228.

TSUKADA, H., KAKIUCHI, T., ANDO, I., SHIZUNO, H., NAKANISHI, S. & OUCHI, Y. (1997) Regulation of cerebral blood flow response to somatosensory stimulation through the cholinergic system: A positron emission tomography study in unanesthetized monkeys. *Brain Research,* 749**,** 10-17.

TSUKADA, H., KAKIUCHI, T., FUKUMOTO, D., NISHIYAMA, S. & KOGA, K. (2000a) Docosahexaenoic acid (DHA) improves the age-related impairment of the coupling mechanism between neuronal activation and functional cerebral blood flow response: a PET study in conscious monkeys. *Brain Research,* 862**,** 180-186.

TSUKADA, H., SATO, K., KAKIUCHI, T. & NISHIYAMA, S. (2000b) Age-related impairment of coupling mechanism between neuronal activation and functional cerebral blood flow response was restored by cholinesterase inhibition: PET study with microdialysis in the awake monkey brain. *Brain Research,* 857**,** 158-164.

TURNBULL, T., CULLEN-DRILL, M. & SMALDONE, A. (2008) Efficacy of Omega-3 Fatty Acid Supplementation on Improvement of Bipolar Symptoms: A Systematic Review. *Archives of Psychiatric Nursing,* 22**,** 305-311.

TURNER, N., ELSE, P. L. & HULBERT, A. J. (2003) Docosahexaenoic acid (DHA) content of membranes determines molecular activity of the sodium pump: implications for disease states and metabolism. *Naturwissenschaften,* 90**,** 521-523.

ULMANN, L., MIMOUNI, V., ROUX, S., PORSOLT, R. & POISSON, J. P. (2001) Brain and hippocampus fatty acid composition in phospholipid classes of aged-relative cognitive deficit rats. *Prostaglandins Leukot Essent Fatty Acids,* 64**,** 189-95.

ULUDAG, K., DUBOWITZ, D. J., YODER, E. J., RESTOM, K., LIU, T. T. & BUXTON, R. B. (2004) Coupling of cerebral blood flow and oxygen consumption during physiological activation and deactivation measured with fMRI. *Neuroimage,* 23**,** 148-155.

UMHAU, J. C., ZHOU, W. Y., CARSON, R. E., RAPOPORT, S. I., POLOZOVA, A., DEMAR, J., HUSSEIN, N., BHATTACHARJEE, A. K., MA, K. Z., ESPOSITO, G., MAJCHRZAK, S., HERSCOVITCH, P., ECKELMAN, W. C., KURDZIEL, K. A. & SALEM, N. (2009) Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. *Journal of Lipid Research,* 50**,** 1259-1268.

VAN BREUKELEN, G. J. P. (2006) ANCOVA versus change from baseline had more power in randomized studies and more bias in nonrandomized studies. *Journal of Clinical Epidemiology,* 59**,** 1334-1334.

VAN DE REST, O., GELEIJNSE, J. M., KOK, F. J., VAN STAVEREN, W. A., DULLEMEIJER, C., OLDERIKKERT, M. G., BEEKMAN, A. T. & DE GROOT, C. P. (2008) Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology,* 71**,** 430-8.

VAN GELDER, B. M., TIJHUIS, M., KALMIJN, S. & KROMHOUT, D. (2007) Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr,* 85**,** 1142-7.

VANCASSEL, S., DURAND, G., BARTHELEMY, C., LEJEUNE, B., MARTINEAU, J., GUILLOTEAU, D., ANDRES, C. & CHALON, S. (2001) Plasma fatty acid levels in autistic children. *Prostaglandins Leukot Essent Fatty Acids,* 65**,** 1-7.

VANDERHASSELT, M. A., DE RAEDT, R. & BAEKEN, C. (2009) Dorsolateral prefrontal cortex and Stroop performance: Tackling the lateralization. *Psychonomic Bulletin & Review,* 16**,** 609-612.

VILLRINGER, A. & DIRNAGL, U. (1995) Coupling of Brain Activity and Cerebral Blood-Flow - Basis of Functional Neuroimaging. *Cerebrovascular and Brain Metabolism Reviews,* 7**,** 240-276.

VOIGT, R. G., LLORENTE, A. M., JENSEN, C. L., FRALEY, J. K., BERRETTA, M. C. & HEIRD, W. C. (2001) A randomized, double-blind, placebo-controlled

trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. *Journal of Pediatrics,* 139**,** 189-196.

VON SCHACKY, C. (2000) n-3 Fatty acids and the prevention of coronary atherosclerosis. *American Journal of Clinical Nutrition,* 71**,** 224S-227S.

VREUGDENHIL, M., BRUEHL, C., VOSKUYL, R. A., KANG, J. X., LEAF, A. & WADMAN, W. J. (1996) Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons. *Proceedings of the National Academy of Sciences of the United States of America,* 93**,** 12559-12563.

WARD, O. P. & SINGH, A. (2005) Omega-3/6 fatty acids: Alternative sources of production. *Process Biochemistry,* 40**,** 3627-3652.

WEBB, G. P. (2006) *Dietary Supplements and Functional Foods,* Oxford, Blackwell Publishing Ltd.

WELCH, A. A., BINGHAM, S. A., IVE, J., FRIESEN, M. D., WAREHAM, N. J., RIBOLI, E. & KHAW, K. T. (2006) Dietary fish intake and plasma phospholipid n-3 polyunsaturated fatty acid concentrations in men and women in the European Prospective Investigation into Cancer-Norfolk United Kingdom cohort. *American Journal of Clinical Nutrition,* 84**,** 1330-1339.

WELCH, A. A., BINGHAM, S. A. & KHAW, K. T. (2008) Estimated conversion of alpha-linolenic acid to long chain n-3 polyunsaturated fatty acids is greater than expected in non fish-eating vegetarians and non fish-eating meat-eaters than in fish-eaters. *J Hum Nutr Diet,* 21**,** 404.

WESNES, K. A., PINCOCK, C., RICHARDSON, D., HELM, G. & HAILS, S. (2003) Breakfast reduces declines in attention and memory over the morning in schoolchildren. *Appetite,* 41**,** 329-331.

WHALLEY, B., JACOBS, P. A. & HYLAND, M. E. (2007) Correlation of psychological and physical symptoms with chronically elevated cytokine levels associated with a common immune dysregulation. *Annals of Allergy Asthma & Immunology,* 99**,** 348-351.

WHICH (2007) Benefit from Omega 3: Confusing Labels. Retrieved on January 15, 2010 from http://www.which.co.uk/advice/omega-3/confusing-labels/index.jsp.

WILLIS, L. M., SHUKITT-HALE, B., CHENG, V. & JOSEPH, J. A. (2008) Dose-dependent effects of walnuts on motor and cognitive function in aged rats. *Br J Nutr***,** 1-5.

WINGEN, M., KUYPERS, K. P. C., VAN DE VEN, V., FORMISAN, E. & RAMAEKERS, J. G. (2008) Sustained attention and serotonin: a pharmaco-fMRI study. *Human Psychopharmacology-Clinical and Experimental,* 23**,** 221-230.

XIAO, Y., WANG, L., XU, R. J. & CHEN, Z. Y. (2006) DHA depletion in rat brain is associated with impairment on spatial learning and memory. *Biomedical and Environmental Sciences,* 19**,** 474-480.

XIAO, Y. F., GOMEZ, A. M., MORGAN, J. P., LEDERER, W. J. & LEAF, A. (1997) Suppression of voltage-gated L-type Ca2+ currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proceedings of the National Academy of Sciences of the United States of America,* 94**,** 4182-4187.

YAO, J. K. & VAN KAMMEN, D. P. (2004) Membrane phospholipids and cytokine interaction in schizophrenia. *Disorders of Synaptic Plasticity and Schizophrenia.* San Diego, Academic Press Inc.

YEHUDA, S., RABINOVITZ, S., CARASSO, R. L. & MOSTOFSKY, D. I. (2000) Fatty acid mixture counters stress changes in cortisol, cholesterol, and impair learning. *International Journal of Neuroscience,* 101**,** 73-87.

YEHUDA, S., RABINOVITZ, S. & MOSTOFSKY, D. I. (1999) Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J Neurosci Res,* 56**,** 565-70.

YOUDIM, K. A., MARTIN, A. & JOSEPH, J. A. (2000) Essential fatty acids and the brain: possible health implications. *Int J Dev Neurosci,* 18**,** 383-99.

ZIMMER, L., DELION-VANCASSEL, S., DURAND, G., GUILLOTEAU, D., BODARD, S., BESNARD, J. C. & CHALON, S. (2000a) Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3 polyunsaturated fatty acids. *Journal of Lipid Research,* 41**,** 32-40.

ZIMMER, L., DELPAL, S., GUILLOTEAU, D., AIOUN, J., DURAND, G. & CHALON, S. (2000b) Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. *Neuroscience Letters,* 284**,** 25-28.

ZIMMER, L., VANCASSEL, S., CANTAGREL, S., BRETON, P., DELAMANCHE, S., GUILLOTEAU, D., DURAND, G. & CHALON, S. (2002) The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. *American Journal of Clinical Nutrition,* 75**,** 662-667.