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AGEING, COGNITION AND OMEGA-3 FATTY ACIDS

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

The evidence for omega-3 polyunsaturated fatty acids (*n*-3 PUFA) in fish oil supplements being a safe therapeutic agent is steadily growing. However, there is still a lack of evidence around the cognitive effects of *n*-3 PUFAs in older adults with Mild Cognitive Impairment (MCI), the moderating effect of the Apolipoprotein E (APOE) ε4 allele on cognition and well-being, and the popularity of fish oil supplements in New Zealand (NZ).

The primary aim of this thesis was to conduct a clinical trial to investigate the cognitive effects of a high dose docosahexaenoic acid (DHA) fish oil supplement in older adults with MCI, and to examine how the presence of the APOE ε4 allele affects the efficacy of fish oil. The trial involved a 12-month randomised, double-blind, placebo-controlled design with testing sessions at baseline, 6-months, 9-months and at the end of the trial. Seventy-two adults with MCI between the ages of 60 and 90 were recruited from Wellington, NZ. Participants were either given a DHA fish oil supplement containing 1491 mg of DHA and 351 mg of eicosapentaenoic acid (EPA) per day or a placebo supplement containing sunflower oil. Outcome measures included the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the National Adult Reading Test (NART), the Coin Rotation Task (CRT), the California Older Adult Stroop Test (COAST), the Trail Making Test A and B (TMT), Digit Span Backwards Test (DSBT), the Geriatric Depression Scale (GDS), the Geriatric Anxiety Inventory (GAI), the 36-Item Short Form Health Survey Questionnaire (SF-36), height, weight and blood pressure as well as red blood cell (RBC) fatty acid profiles. ANCOVAs, *t*-tests and chi-square tests were used to test for differences between the DHA and placebo groups.

The secondary aim of this thesis was to conduct an online cross-sectional survey to

investigate how popular fish oil supplements are within a sample population of New Zealanders, to determine why fish oil supplements are used, what dose fish oil users take, and where fish oil users store their supplements. The aim of the survey was to test the hypothesis that fish oil users are more likely to display healthier dietary and lifestyle habits.

The final analysis (n = 60) of the trial found no evidence of a treatment effect using the cognitive measures, although it did find a treatment effect on systolic blood pressure (p = .03, η^2 = .08), and a treatment interaction for APOE ε4 carriers on depression (p = .04, η^2= .07) and anxiety (p = .02, η^2 = .09) scores in favour of the DHA group. The survey found that in a sample of 334 New Zealanders, 66.8% reported using supplements and 21.9% reported using fish oil supplements. The survey found that respondents who regularly eat oily fish are most likely to take fish oil supplements (p < .01), and that only 26% of fish oil users reported taking a dose that would meet dietary recommendations. Over half of fish oil users reported taking fish oils to 'improve brain functioning' and only 6.8% of fish oil users reported storing fish oil supplements in the refrigerator.

The evidence from epidemiological studies suggests that fish consumption is protective against cognitive decline and dementia, and yet the evidence from clinical trials investigating the cognitive effects of *n*-3 PUFAs in older adults has been inconsistent and at times conflicting. It appears that the protective effect of fish may be more than the *n*-3 PUFA content, and that fish consumption may be part of an overall healthier diet and lifestyle, along with regular physical activity, strong social connections and an emphasis on educational attainment and engagement in cognitive activities.

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List of Abbreviations

AD	Alzheimer's disease
ADAS	Alzheimer's Disease Assessment Scale
AI	Adequate intake
ALA	Alpha-linolenic acid
AMD	Age-related macular degeneration
APOE	Apolipoprotein E gene
ARA	Arachidonic acid
ARCD	Age-related cognitive decline
BDNF	Brain derived neuro-trophic factor
BMI	Body mass index
CDR	Clinical Dementia Rating
CHL	Canterbury Health Laboratories
CIND	Cognitive impairment no dementia
COAST	Californian Older Adult Stroop Test
CRT	Coin Rotation Task
CSF	Cerebrospinal fluid
DHA	Docosahexaenoic acid
DLB	Dementia with Lewy bodies
DSBT	Digit Span Backwards Test
EPA	Eicosapentaenoic acid
FFQ	Food Frequency Questionnaire
FTD	Fronto-temporal dementia
GAI	Geriatric Anxiety Inventory
GDS	Geriatric Depression Scale
HDEC	Health and Disability Ethics Committee
HDL	High-density lipoproteins
HDS-R	Hasegawa's Dementia Rating Scale
IDL	Intermediate-density lipoproteins
LA	Linoleic acid
LDL	Low-density lipoproteins

LOCATO	Visuospatial object location memory task
M	Mean
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MUFA	Monounsaturated fatty acid
MV	Multi-vitamin
NA	Nervonic acid
NART	National Adult Reading Test
NSAID	Nonsteroidal anti-inflammatory drug
NZ	New Zealand
OD	Other forms of dementia
OR	Odds ratio
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
n-9 PUFA	Omega-9 polyunsaturated fatty acid
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
RA	Rheumatoid arthritis
RBC	Red blood cell
RCT	Randomised controlled trial
RDA	Recommended daily allowance
SCL	Southern Community Laboratories
SD	Standard deviation
SDT	Suggested dietary target
SFA	Saturated fatty acid
TMT	Trail Making Test
U3A	University of the Third Age
ULDL	Ultra-low-density lipoproteins
VD	Vascular dementia
VLDL	Very-low-density lipoproteins
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale

Preface

I came to doctoral study after spending 10 years away from academic research. During those 10 years, I studied towards a Diploma in Herbal Medicine in an attempt to find alternative treatments and solutions to the disorders that I had read so much about during my postgraduate study in neuropsychology.

The other major thing that happened in my life during those 10 years was the cognitive decline of my Nana. I was 25 years of age at the time, in the last year of the Diploma, and coincidentally had moved in to live with my Aunty around the same time that my Nana had. This was the year before she went into a specialised aged-care facility, the last year that she was really ever herself. That year we spent hours watching tennis, one of the favourite pastimes of her youth, and talking about cooking and her days as a nurse and missionary in the Solomon Islands. One day I went into her room to see her still lying in bed. She looked very confused and was holding her head as if she had a headache. I asked if she was okay and she told me she wasn't. She asked me to call an ambulance. When the ambulance officer came, he asked her a few questions, examined her, and then eventually recommended that she go into hospital for a few tests. The team at the hospital found that she had had several small strokes – things changed from that moment on.

After her diagnosis of vascular dementia, the topic of treatment and medication was explored, and my family asked whether there was anything else, any natural supplements that could help. During my herbal medicine training, I had learnt about the use of *Ginkgo biloba* as a cognition enhancer with anti-platelet effects to help reduce further strokes. Despite a long history of use, I knew that the results from clinical trials with Ginkgo in older adults with Alzheimer's disease were inconsistent – some positive, some negative. If there was to be a benefit, would it outweigh the financial cost and the burden of the ever-

increasing number of pills she had to take each day? The answer turned out to be no. She made the decision to move into an aged-care facility that same year, and 3 years later, she moved into a specialised dementia unit. She died about a year later.

Around the time she passed away, I began to read about the link between the *n*-3 PUFAs in fish and fish oil supplementation and cognition, both prenatally and in older adults. I saw that this was where I could begin my research into a 'solution'. I approached my supervisor and after a literature review realised that somewhere between normal ageing and dementia was where fish oil supplementation may have the most beneficial effect. I decided that the greatest contribution I could make with my knowledge and skills would be with a clinical trial, and so settled on using a randomised, placebo-controlled method to investigate the cognitive effects of fish oil supplementation in older adults.

One of the last things my Nana said to me was "Don't worry dear, I know who I am and I know who the Lord is". She knew what was happening to her and she also knew what was important to her. However, she was vulnerable, and needed caring and trustworthy people to ensure her the dignity she deserved. She was exactly what you would want in a Nana – patient, caring and with biscuit tins always full. She taught me two very important things in my life – how to cook and how to be resilient, although she would have called it toughness – a quality that I believe has led me to where I am now. Researching preventive measures for severe cognitive decline is only a small part of the work that is needed in the area of dementia research, how we care for those who are already affected is also of utmost importance.

Thesis Overview

The treatment and prevention of dementia has become one of the most challenging issues facing health professionals and researchers today. Alzheimer's Disease International estimated that in 2016 approximately 47 million people worldwide were living with dementia, with this number expected to increase to more than 131 million by 2050 (Prince, Comas-Herrera, Knapp, Guerchet, & Karagiannidou, 2016). The identification of a transitional state between normal age-related cognitive change and dementia, termed Mild Cognitive Impairment (MCI), has helped to shed light on the risks and likelihood of developing dementia in later years (Petersen et al., 2009). Focusing specifically on older adults with MCI provides researchers with an opportunity to investigate the effects of lifestyle factors such as diet on cognition at the pre-symptomatic stage of dementia.

Nutritional research has shown that most older adults are not eating enough fish and seafood to reach recommended daily amounts of docosahexaenoic acid (DHA) (Bourre & Paquotte, 2008; Cole & Frautschy, 2010) and at the same time epidemiological research has shown positive associations between fish consumption and both higher scores in cognitive tests (e.g., Dangour et al., 2009) and a slower rate in cognitive decline (e.g., Morris, Evans, Tangney, Bienias, & Wilson, 2005). DHA and eicosapentaenoic acid (EPA) are *n*-3 PUFAs found in most types of fish and seafood, although in higher amounts in oily fish such as salmon, sardines and mackerel. These two fatty acids are commonly referred to as 'conditionally essential fatty acids' because the human body cannot easily convert the plant-derived precursor alpha-linolenic acid (ALA) to DHA, and therefore, for most people EPA and DHA must be consumed in the diet or taken as a supplement. As well as being important for reducing inflammation and blood clotting, DHA in particular is crucial for the integrity of

neuronal cell membranes, for neuronal cell signalling, as well as providing a protective mechanism against neuro-toxicity (Salem, Litman, Kim, & Gawrisch, 2001).

The extent to which lifestyle or environmental factors and genetics affect the progression from healthy age-related cognitive functioning to dementia remains unclear. The interplay between genes and nutrition in the development of dementia provides an interesting platform to examine the effects of a particular gene on the efficacy of a specific nutrient. The primary aim of this thesis was to address this issue by investigating the effects of a high-dose DHA fish oil supplement on cognitive performance and well-being in older adults with MCI, and by examining how the presence of the Apolipoprotein E (APOE) ε4 allele affects the efficacy of fish oil. The APOE gene plays an important role in the transport of fats and cholesterol within the brain, and the presence of the APOE ε4 allele has been shown to increase the likelihood of developing Alzheimer's disease (Barberger-Gateau, Samieri, Feart, & Plourde, 2011). There is enormous potential for research into gene-nutrient interactions to help develop targeted treatments that can prevent the onset of dementia.

The research presented in this thesis examines the link between fish oil supplementation, the APOE ε4 allele and cognitive performance in older adults using a randomised, double-blind, placebo-controlled intervention trial (RDBPC) or randomised controlled trial (RCT) – considered the gold standard in clinical studies. Despite the increasing popularity and use of complementary medicines, there is still considerable scepticism about the therapeutic value and use of these products. This scepticism is largely due to a lack of scientific research to back up the efficacy and safety of these products, but also a general lack of information about what products and dose are best suited to particular medical conditions. The majority of complementary medicines are purchased and consumed

without professional advice, and some products are still manufactured outside the good manufacturing practices framework, which controls the manufacture and sale of food, drugs and pharmaceutical products in New Zealand (NZ).

Nutritional supplements are increasingly being used by older adults, particularly fish oil, vitamin D, glucosamine and coenzyme Q10, as older adults search for alternatives to pharmaceutical drug treatments and preventative solutions to age-related conditions such as age-related macular degeneration, arthritis and cognitive decline (Conference on Dietary Supplement Use in the Elderly, 2004). With this increased use, there is a need to understand the pharmacology, toxicology, the benefits vs. harmful aspects of these products, as well as quality-related issues like sourcing of the ingredients and contraindications with pharmaceutical medications. The secondary aim of this thesis was to conduct an online cross-sectional survey to determine what percentage of a sample of New Zealanders use fish oil supplements, why people take fish oil supplements, where fish oil supplements are stored, the average daily dosage, and to examine what health behaviours are associated with fish oil supplement use. The survey was also used to see how willing people were to have a blood sample taken as part of a research study to assess this as a part of the exclusion criteria for the trial.

This thesis is divided into 9 chapters. Chapter 1 begins the literature review with an explanation of current ageing theories, and then examines the terminology and theories used in cognitive ageing from normal cognitive ageing to age-related cognitive decline (ARCD), Cognitive impairment no dementia (CIND) and MCI through to dementia. The chapter ends with a brief description of the current pharmaceutical treatments available for dementia. Chapter 2 reviews the negative and positive risk factors for cognitive decline in older adults in order to understand how important diet and nutrition are in reducing the risk

for cognitive decline and dementia. Chapter 3 examines the chemistry, metabolism and pharmacological effects of *n*-3 PUFAs before reviewing the epidemiological evidence and mechanisms for the cognitive effects of EPA and DHA in older adults. A decision was made to exclude, as much as possible, clinical trials and reviews, which involved animals or animal models. The main reason why researchers are interested in the effects of EPA and DHA is that the human body cannot easily produce EPA and DHA, whereas most other animals can (Russo, 2009). It is of limited use to involve animals in research to understand human fatty acid metabolism, DHA homeostasis and the effects of DHA on cognition when these animals can convert these fatty acids while humans cannot, and especially when fish oil supplementation has such a good safety record.

Chapter 4 contains the manuscript of the fish oil supplement survey, which includes information about current dietary recommendations for EPA and DHA as well as issues related to oxidised fish oil supplements. Chapter 5 reviews the most relevant RCTs that have investigated the effects of fish oil supplementation on cognitive performance in cognitively healthy older adults, older adults with MCI and older adults with dementia. Chapter 6 introduces the objectives, research questions, and hypotheses of the current fish oil RCT, highlighting areas of design originality and significance. Chapter 7 outlines the method of the RCT in detail, in particular, the inclusion and exclusion criteria for participants, screening and recruitment, the cognitive and well-being measures and the blood analysis procedures. The trial method and a brief literature review were published in a Massey University Manawatu Doctoral Research Symposium – Mengelberg, A., Leathem, J., Podd, J., & Conlon, C. (2018). Investigating the effects of a DHA fish oil supplement on cognitive performance in older adults. *Refereed Proceedings of Doing Psychology: Manawatū Doctoral Research Symposium*, *3*, 25-31. Chapter 8 presents the statistical analysis and results of the trial, and

finally, Chapter 9 discusses the results of both the RCT and survey in terms of the implications for the use of fish oil supplements in preventing cognitive decline as well as the limitations of the trial and future recommendations.

CHAPTER 1

AGEING AND COGNITION

Chapter 1 begins with a description of current ageing theories, both biological and psychological, to understand why some older adults develop severe forms of cognitive decline as they age while others do not. The chapter then moves on to examine the terminology and theories of cognitive ageing in more depth, from age-related cognitive decline to severe forms of cognitive decline such as dementia, including Alzheimer's disease (AD) as well as milder forms such as Mild Cognitive Impairment (MCI) in an attempt to understand what stage of cognitive decline may be the best time to intervene with preventive strategies. The chapter ends with a brief description of the current pharmaceutical treatments available for dementia.

1.1 Ageing theories

Several ageing theories have been proposed to help explain the physical and biological changes that take place during a person's lifetime. The first group of theories are based on error theory, which holds that ageing is the result of environmentally caused errors and damage, which accumulate over the years. The first of these, the wear and tear theory proposed by August Weisman in 1886, is based on the idea that the 'wear' and 'tear' from internal (cellular and molecular) and environmental (radiation and toxins) factors is what

causes ageing (Salvi, Akhtar, & Currie, 2006). Another based on error theory, the free radical theory, was introduced by Rebeca Gerschman in 1954 and further developed by Denham Herman in 1956. It states that normal physical functioning, such as energy metabolism and respiration, results in a series of chain reactions that creates free radicals, causing damage to cells (Harman, 1992). The body's ability to offset the oxidative damage with free radical agents and anti-oxidants is reflected in the extent of cellular damage over a lifespan. A third error-based theory, the cross-linking theory proposed by Johan Bjorksten in 1942 states that ageing is the result of an accumulation of large cross-linked or aggregated molecules (tangles) which disrupt vital processes and cause damage to cells (Bjorksten & Tenhu, 1990). Lastly, the DNA damage theory proposes that throughout life as DNA is copied and replicated within cells, errors occur resulting in mutations and eventually damage to cells (Jin, 2010).

The next group of ageing theories are based on a biological clock or programmed idea, that ageing is a result of programmed changes within genetic material. The first of these, the endocrine theory, states that ageing is the result of a reduction in neurotransmitter and hormone production such as insulin and thyroid-stimulating hormone, which causes dysfunctions in metabolism, sleep, appetite and libido (Meites, Goya, & Takahashi, 1987). The immune system theory proposed by Roy Walford in 1964 states that ageing is the result of a decline in the function of the immune system, resulting in autoimmune reactions, a reduction in the production of anti-bodies and increased levels of inflammation (Walford, 1964). The genetic or programmed longevity theories state that ageing is due to the stability of genes throughout the lifespan, particularly the switching on and off of specific genes (in some people but not others) as well as specific genetic variations (Davidovic et al., 2010). Finally, the telomere theory states that each time a cell

divides the telomeres (the cap at the end of each DNA strand) shorten, which causes disruptions in homeostatic mechanisms and the lifespan of cells (Olovnikov, 1996).

Several psychological theories have been proposed to help explain the changes in mental health, emotional well-being and cognition with age. One of the earliest is the disengagement theory of ageing proposed by Cumming and Henry in 1961 which states that as people age, they withdraw from society due to a reduced capacity, as well as in order for the younger generations to move forward (Achenbaum & Bengtson, 1994). In contrast, theories such as the activity theory state that active engagement, both physically and socially, is what contributes to successful ageing (Havighurst, 1968), and the continuity theory, which asserts that older adults usually maintain the same level of activity and interaction in later years by using adaption strategies (Atchley, 1989). This ability to adapt with age has been the focus of an area of research called 'positive and successful ageing'. The selection, optimization and compensation theory of successful ageing states that older adults 'select' by identifying relevant goals and prioritising them, 'optimise' by choosing strategies to successfully achieve goals and 'compensate' by adapting to limitations (Baltes, 1997). In terms of social support, the convoy theory specifies that there are three key benefits to social support with age; firstly as 'aid' or for instrumental support, secondly as 'affect' or for emotional support, and thirdly as 'affirmation' for shared goals and values (Antonucci & Akiyama, 1987).

No single ageing theory has been able to explain all of the mechanisms or processes involved in ageing. It is most likely that a combination of theories is best at explaining why some older adults show signs of ageing earlier or develop cognitive impairments and others do not. Several of these ageing theories have now been supported by research, for example some individuals have been shown to have telomeres that increase in length (rather than

shorten) with cell division, and some studies have reported that inheriting shorter telomeres is associated with an increased risk of coronary artery disease (e.g., Brouilette et al., 2008). Understanding the role and importance of social support with age is also gaining momentum with recent research showing a strong link between loneliness and impaired cognition (e.g., Wilson et al., 2007; O'luanaigh et al., 2012). The role of oxidative stress and inflammation in the pathology of cognitive decline in ageing is not fully understood, although there is support for the role of oxidative damage, including increased free radical production and increased lipid peroxidation, in AD pathology (Floyd & Hensley, 2002). The role of genetics in cognitive decline, especially in early-onset AD, is firmly established, whereas the central etiological hypothesis for late-onset AD is for a genetic and environmental interaction (Barberger-Gateau et al., 2011).

1.2 Normal ageing, the brain and cognition

The term 'normal ageing' refers to patterns of cognitive change, which are commonly found in human ageing (Atchley, 1989). 'Cognitive ageing', 'age-related cognitive change' and 'age-related cognitive decline' (ARCD) all refer to the cognitive changes which take place with ageing. ARCD begins as early as in the 20s, particularly in processing speed, reasoning and spatial orientation (Salthouse, 2009). However, the focus here will be on the structural, functional and cognitive changes associated with ageing from midlife onwards. In order to investigate the relationship between neuroanatomical and cognitive changes with age, researchers have used structural and functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), often by comparing young to older adults and by using both cross-sectional and longitudinal methods. The question of whether specific age-related

structural changes cause the age-related cognitive changes is not clear, although several clear age-related trends have been identified (Kennedy & Raz, 2009).

The first age-related change in the brain is the slowdown in neurotransmitter production and transportation, and the reduction in receptor sensitivity, particularly with the neurotransmitter acetylcholine and the choline receptors (Perry, 1980). Levels of dopamine and serotonin transportation also decrease with age, affecting cognition and emotional well-being (Kuikka et al., 2001; Karrer et al., 2017). Secondly, there are several significant structural changes in the brain. These include a shrinkage of overall brain volume (due to the shrinking volume of neurons), changes to white matter fibres (reduced myelinated axon length), and changes in regions such as temporal lobe, particularly to the hippocampus which can cause memory impairments and a decline in visuospatial abilities (Fjell & Walhovd, 2010). The hippocampus, a subcortical structure within the medial temporal lobe, primarily involved in the consolidation of short-term to long-term memory and in spatial awareness and emotion, decreases in size in normal ageing but significantly more so in those with dementia. The hippocampus has been shown to be vulnerable to internal and external influences such as levels of cortisol and inflammatory mediators, exercise, sleep and alcohol (Fotuhi, Do, & Jack, 2012). Changes to white matter (axons and glial cells) and the callosal fibres affect the connections between regions of the brain, which can impair the ability to function in everyday activities. As well as deficits in memory, particularly in episodic and spatial memory, and processing speed, there is also a decline in parts of executive functioning with increasing age.

Several types of cognitive theories have been proposed to help explain these age-related cognitive changes. Attention and processing speed theories propose that the reduction in processing speed (how quickly information can be understood and utilised)

underlines the deficits found in cognition functioning in the later years (Salthouse, 1996). Support for the processing speed theory is based on the finding that measures of processing speed share approximately 75% of the age-related variance with other cognitive measures such as memory including working memory (Salthouse, 1996; 2000). Executive function theories propose that components of executive functioning such as attention, inhibition and the role of the frontal lobe in control, coordination and compensatory mechanisms, help explain age-related cognitive decline (e.g., Phillips & Henry, 2008). Attention and processing resource capacity theories suggest that cognitive decline is due to attention deficits as a result of limited processing resources, whereas inhibition theories suggest that deficits in the ability to inhibit information increase with age resulting in forgetfulness and distractibility (Craik & Byrd, 1982; Hasher & Zacks, 1988). Structural and functional changes within the frontal lobes with increasing age have been used to explain the lower performance of older compared to younger adults in cognitive tasks requiring control, coordination, integration and goal maintenance (West, 1996).

Support for executive functioning-based theories alone is limited, although when combined as a multivariate approach with processing speed theories and temporal lobe-based memory deficits, they have been able to account for a larger percentage of age-related variance in cognition (Albinet, Boucard, Bouquet, & Audiffren, 2012). The idea that multiple approaches, factors or regions contribute to age-related cognitive change forms the basis of compensatory theories. One such compensatory theory is the scaffolding theory of ageing and cognition, which states that the brain can compensate for the deterioration in certain areas by recruiting from brain regions that are still intact (Reuter-Lorenz & Park, 2014). This theory is based on the idea that the changing brain is seeking a state of homeostasis or equilibrium in cognitive functioning by strengthening existing connections or

by developing new alternative connections. In a verbal vs. spatial working memory task using PET, young adults were shown to use predominantly the left hemisphere for the verbal task and the right hemisphere for the spatial task, whereas older adults were shown to engage both regions during both types of tasks (Reuter-Lorenz et al., 2000). The ability to utilise multiple regions of the brain, while younger adults typically use one or two to complete the same task, and to utilise the same brain regions but in different ways, also helps to explain why older adults typically perform better at complex tasks requiring diverse perspectives or contradictory information (e.g., Cabeza, Anderson, Locantore, & McIntosh, 2002; Stern et al., 2005; Spreng, Wojtowicz, & Grady, 2010).

As well as structural changes within the brain, several normal age-related changes outside of the brain have been shown to have secondary effects on cognition. With age the heart muscle begins to thicken, and arteries begin to stiffen and narrow, thereby reducing the pumping rate and increasing blood pressure. This can result in a reduction in blood flow to the brain, and if combined with low clotting factors, can cause severe blockages in cerebral arteries, resulting in a stroke (Breteler et al., 1994). Depending on the level of severity and where in the brain the blockage occurs, the aftereffects can be a physical or cognitive weakness, a loss of sensation, difficulty speaking, talking and walking; and these effects can be either short or long-term. Having high systolic blood pressure, a high body mass index (BMI) and smoking cigarettes are risk factors for a stroke, which highlights the link between lifestyle choices and brain health (Asplund et al., 2009). Muscle tone and bone density also reduce with age, resulting in decreased mobility, an increase in the incidence of falls and a reduction in exercise, which impacts on well-being and cognitive health (Daley & Spinks, 2000). Changes to sensory systems also occur with age, particularly to vision and hearing, which can impair cognitive input and brain stimulation as well as the capacity for

social interaction and independence (Lin et al., 2004). Further, having multiple chronic conditions or multi-morbidity together with the use of multiple pharmaceutical treatments, is increasingly common with older age, and can significantly affect cognition and quality of life.

1.3 Severe cognitive decline and dementia

Dementia is the syndrome most associated with a state of severe cognitive decline. The term dementia is used to describe a group of conditions that involve a cognitive deficit, usually in memory plus at least one other cognitive domain such as language, along with personality and sensory changes (Winblad et al., 2016). Dementia is characterised by a level of cognitive decline that significantly changes or interferes with a person's ability to perform everyday activities (Blennow, Leon, & Zetterberg, 2006). In 2016 approximately 62,000 New Zealanders had a form of dementia, with a global figure of approximately 47 million set to reach 131 million by 2050 (Alzheimers New Zealand, 2016; Prince et al., 2016). These figures highlight the urgent need to understand the pathological mechanisms underlying dementia to develop preventative and treatment measures.

Alzheimer's disease (AD) is the most common cause of dementia and accounts for approximately 60% of all dementia cases (Winblad et al., 2016). Vascular dementia (VD), the second most common cause, is due to a series of strokes as a result of a blockage or the bursting of cerebral blood vessels (van der Flier & Scheltens, 2005). The other main causes and subtypes of dementia include dementia with Lewy bodies (DLB) which includes Parkinson's disease dementia, Huntington's disease (HD) and Fronto-temporal dementia (FTD) also known as Pick's disease or frontal lobe dementia. The initial cognitive impairments of older adults with AD are typically in episodic memory, particularly delayed

recall, with poor retrieval on recognition tests, followed by general knowledge amnesia and aphasia with poor category and verbal letter fluency (Salmon & Bondi, 2009). In comparison, the initial cognitive deficits seen in VD are in executive functioning and episodic memory. In DLB the impairment is in learning new information rather than in retention and recognition, in HD the deficit is in general retrieval and attention, and in FTD it is less of a memory deficit, more so a deficit in verbal fluency particularly for letter fluency alongside major personality and behavioural changes (Salmon & Bondi, 2009).

Because AD is the most common cause of dementia, it has been the most intensively studied in terms of pathology, and causal and modifying factors. The pathology of AD involves the development of neurofibrillary tangles made up of hyper-phosphorylated tau on the microtubules of axons, and the development of senile or neuritic plaques made of amyloid β protein fragments between neurons (Blennow et al., 2006). Both tau and amyloid β are intrinsic to the brain – tau is a normal axonal protein and amyloid β is produced during normal cell metabolism; however, malfunctions in tau and amyloid β cause severe neuronal degeneration (Himmelstein, Ward, Lancia, Patterson, & Binder, 2012). The tangles from the hyper-phosphorylation of tau impair nutrient transport and axonal function, and the imbalance between the production and clearance of amyloid β causes plaques to form, which blocks synapse signals and causes an immune response that triggers inflammation (Kim & Tsai, 2009). The tangles and plaques cause disruptions in cell-to-cell communication, which eventually results in neuronal atrophy or neuron cell death (Blennow et al., 2006). This neuronal atrophy initially affects the medial temporal lobe and the hippocampus, and then eventually the cortical areas; this leads to a loss in functional connectivity, which impairs interaction between regions and systems of the brain (Salmon & Bondi, 2009).

Several hypotheses have been proposed to explain the etiology of dementia and AD.

These include a genetic susceptibility, vascular and cardiovascular risk factors (including cholesterol homeostasis), lifestyle factors during midlife, and a disruption to inflammatory and oxidative processes (Lin, Chiu, Huang, & Su, 2012). In early-onset forms of AD, when pathology develops by middle age, genetic mutations account for the majority of cases (Prince, 2007). In contrast, late-onset sporadic AD appears to have a multifactorial etiology with the exact causes unknown, but most likely, is due to a combination of ageing processes with genetic and environmental or lifestyle factors (Winblad et al., 2016). For example, a large health and ageing study based in Canada involving 740 older adults over the age of 65 found that the highest prevalence and incidence of dementia was in those who had a stroke and who were carriers of the APOE ε4 allele (Jin, Østbye, Feightner, Legge, & Hachinski, 2008). The authors note that the effect of having a stroke was unmodified by APOE status, therefore implying that the two factors led to dementia byway of different mechanisms.

The pathology and neurodegeneration behind dementia is estimated to start between 10 to 20 years before any clinical symptoms appear (van der Flier & Scheltens, 2005; Dubois et al., 2016) and is termed the preclinical phase (Hubbard, Fenton, & Anderson, 1990). During this phase, damage caused by the disease process is thought to take place until a certain threshold is reached (such as a loss of a critical volume of neurons) and the first symptoms appear (Blennow et al., 2006). The ability to detect whether someone is in the preclinical stage of dementia using biological markers and with neuropsychological assessment has been the focus of extensive research (e.g., Elias et al., 2000; Jack Jr et al., 2013). Researching the pathological processes involved during this preclinical phase has provided a deeper understanding of how cognition declines with age, although deciphering what is normal age-related change from age-related pathological

processes has presented a significant challenge within cognitive ageing research (Fjell & Walhovd, 2010).

In addition to the preclinical phase, several other stages have been identified in the progression from 'normal cognition' to dementia. Two 7-stage systems called the Global Deterioration scale and the Functional Assessment Staging (FAST) were developed by Reisberg in 1982 and 1988 to describe the stages of dementia and AD (Sclan & Reisberg, 1992). Stage 1 is classified as 'no impairment' or 'no cognitive decline' where no detectable signs or symptoms of dementia or AD are evident, stage 2 as 'very mild cognitive decline' where minor subjective memory complaints are present, but with no detectable changes on cognitive tests or by family. Stage 3 is classified as 'mild cognitive decline' where friends and family start to notice memory and cognitive lapses, particularly with remembering names of newly met people and finding the right word. Stage 4 as 'moderate cognitive decline' where clear AD symptoms are present such as poor short-term memory like forgetting what the person had for breakfast. Stage 5 is classified as 'moderately severe cognitive decline' where the person begins to experience confusion and needs help with everyday activities. Stage 6 is classified as 'severe decline' where there are personality changes, 'problematic' behaviour problems such as wandering, and when the person can no longer remember their own personal history. Stage 7 is 'very severe cognitive decline' where the person has lost the ability to communicate and respond to their environment, and has lost insight into their condition. Exactly what factors explain why a person moves from one stage to another, and how long it takes to move through these stages, varies enormously from person to person.

1.4 Mild forms of cognitive impairment

The need for an identifiable state of cognitive change or decline between normal ageing and dementia resulted in the development of several terms (along with diagnostic criteria) including Questionable Dementia (QD) based on a score of 0.5 on the Clinical Dementia Rating scale (CDR; Hughes, Berg, Danziger, Coben, & Martin, 1982), Age-Associated Cognitive Decline (AACD; Levy, 1994), Mild Neurocognitive Disorder from the DSM-IV (APA, 1994), Cognitive impairment with no dementia (CIND; Ebly, Hogan, & Parhad, 1995), Mild Cognitive Impairment (MCI; Flicker, Ferris, & Reisberg, 1991; Smith et al., 1996) and Minor Neurocognitive Disorder from the DSM-V (APA, 2013). MCI is the term most widely used to describe this intermediate cognitive state, and is the term that will be used in this thesis.

The term MCI evolved to describe an older adult who scored 3 or had reached stage 3 on the Global Deterioration Scale and FAST 7-stage systems (Sclan & Reisberg, 1992). Stage 3 in these systems specifies a level of mild cognitive decline when the earliest signs of a cognitive deficit appear. The term MCI was then adopted by Petersen and colleagues in 1999 to outline a criteria for the diagnosis of MCI. These criteria specified a diagnosis of MCI as the presence of a memory complaint, a normal ability to carry out activities of daily living, normal general cognitive function, but abnormal memory for age, and not demented. The definition of MCI was then further expanded to include selective impairments in cognitive domains other than memory, the differentiation between amnestic MCI (primarily a memory dysfunction) and non-amnestic MCI (primarily a language, attention, spatial and/or executive dysfunction) as well as the classification of single and multiple domain subtypes to correspond with the different dementia types and etiologies (Petersen, 2004; Petersen, & Morris, 2005; Busse, Hensel, Gühne, Angermeyer, & Riedel-Heller, 2006).

Table 1. Criteria for Mild Cognitive Impairment from Petersen (2004) and Albert et al. (2011)

1. Presence of a memory or cognitive complaint
2. An objective impairment in at least one cognitive domain
3. Preserved general cognitive function
4. A preserved ability to carry out everyday activities
5. No dementia

Table 1 outlines the most common components of current MCI criteria. In terms of point 1, more recent criteria have emphasised that there needs to be a level of concern in regards to the recent cognitive complaint or change from the individual, which is then confirmed by a family member or friend (Albert et al., 2011). Point 2 has been quantified to be assessed as a score or scores on cognitive tests of between 1 to 1.5 standard deviations (SD) below the mean for age, to be used as a guideline but not necessarily as a cut-off (Albert et al., 2011). Point 3 is the point most likely omitted from an MCI criterion. In 2004 Petersen added the term 'relatively' to 'preserved general cognitive function' for age, which reflects the ongoing problem with differentiating a 'mild' from a 'very mild' cognitive impairment. Other MCI criteria have stressed the need to rule out other trauma, medical conditions and brain diseases that could explain the cognitive decline such as a head injury or stroke, as well as the inclusion of diagnostic biomarkers such as cerebrospinal fluid (CSF) levels of amyloid β 1 (Albert et al., 2011; Petersen et al., 2018).

The amendments made to the MCI criteria over time are the result of how MCI has been conceptualised and re-conceptualised over the years (Petersen et al., 2018). MCI has been described as the transitional stage between normal ageing and dementia, as a prodromal state of dementia, as part of the preclinical phase before any clinical symptoms

of dementia appear, as a stage distinct from the preclinical phase, as a risk factor (not a state or condition) of dementia, and as a benign form of the normal ageing process (e.g., Flicker, Ferris, & Reisberg, 1991; Tuokko & Frerichs, 2000; Ganguli et al., 2004; Albert & Blacker, 2006; Gauthier et al., 2006; Fischer et al., 2007; Petersen et al., 2009). The issues and controversy surrounding MCI are primarily the result of the criteria being implemented in different ways in clinical vs. research settings, and in part, due the wide range of symptom severity, etiologies and trajectories (Albert & Blacker, 2006; Petersen et al., 2009; Stephan et al., 2013). To increase the diagnostic reliability and validity of MCI, further variations have been proposed including 'MCI due to AD' along with levels of certainty (e.g., intermediate likelihood, high likelihood or unlikely due to AD), based on the presence or absence of certain biomarkers (Albert et al., 2011). Whether any of these variations have resulted in a clearer definition, criteria or improved predictive power is unclear.

The concept of MCI as the earliest detectable stage of a clinical impairment has remained and as a research entity, has been utilised to estimate conversion rates from MCI to dementia, to predict clinical outcomes and to investigate risk factors and possible preventative treatments for dementia (Winblad et al., 2004). Research indicates that individuals who receive a diagnosis of MCI are at an increased risk for developing dementia (Petersen et al., 1999), although annual conversion rates vary from as little as 1 to 3% (Fisk, Merry, & Rockwood, 2003), 5 to 10% (Mitchell & Shiri-Feshki, 2009), between 10 to 15% (Ganguli et al., 2004; Petersen et al., 2018) and up to 26.8% in non-amnesic MCI and 48.7% in amnestic MCI (Fischer et al., 2007). The variability appears to be somewhat based on the type of sample, for example, Farias et al. (2009) found an annual conversion rate of 3% for a community based sample (community health care facilities, senior and church groups), yet a rate of 13% for a clinic sample (university-based memory clinic). Busse et al. (2006) found

that conversion rates varied from 9 to 42% depending on whether a 1 or 1.5 SD cut-off criteria was used for assessing the cognitive impairment. Studies have also differed in the number of older adults with MCI who improve to a normal status, from 15 to 44% (Fisk et al., 2003), 20% (Busse, Bischkopf, Riedel-Heller, & Angermeyer, 2003) and from 33 to 55% who no longer had MCI (Ganguli et al., 2004).

Assessing the cognitive impairment component of MCI has been problematic for researchers both in terms of identifying the most sensitive cognitive measures and the most appropriate cut-off point. There is increasing evidence to support the idea that measuring memory deficits alone is insufficient, whereas measuring memory deficits alongside secondary or non-memory deficits, especially in attention, processing speed and executive functioning are more predictive of cognitive decline (Traykov et al., 2007; Saunders & Summers, 2011). The cognitive tests shown to be best at discriminating between MCI, AD and cognitively healthy older adults are a combination of a word list delayed recall test with the Trail Making Test B (Chen et al., 2000), immediate and delayed free recall in combination with the Stroop Test (Traykov et al., 2007), and error scores particularly in executive functioning based tasks like the Tower of London (Rainville, Lepage, Gauthier, Kergoat, & Belleville, 2012), and the incongruent component of the Stroop Test (Bélanger, Belleville, & Gauthier, 2010).

The cut-off point for the cognitive deficit is also an important factor, with studies showing that a cut-off of 1 SD results in higher sensitivity but lower specificity, while a cut-off of 1.5 SDs results in the opposite (Busse, Bischkopf, Riedel-Heller, & Angermeyer, 2003). Most cognitive tasks and therefore cut-off points are assessed according to age norms, whereas it has been suggested that education norms or educational background are just as important (Albert et al., 2011). Highly educated individuals pose a significant problem for

MCI research, as scores within the `normal' range could mask a decline from the individual's pre-morbid ability. The inclusion of pre-morbid functioning measures such as the National Adult Reading Test (NART) allows the clinician or researcher to determine whether scores on cognitive tasks represent a decline from the premorbid level of functioning (Ahl et al., 2013). Whether the cognitive deficit is in a memory or a non-memory task, and whether the impairment is in one or at least two tasks or domains are also decisions that need to be made. Considering there is currently no set way to operationalise MCI and with the many variations of MCI criteria and cognitive tasks used in research, particularly in clinical trials, comparing the results from trials can be very challenging.

1.5 Current treatments for dementia

There are four main types of pharmaceutical treatments used in older adults with moderate to severe cognitive decline. These include symptomatic treatments, AD modifying treatments, anti-inflammatory treatments and cholesterol lowering treatments.

Symptomatic treatments help provide symptomatic improvement in older adults with dementia at both a behavioural and cognitive level, but do not significantly alter the pathology or the course of the disease. The first type of symptomatic treatment involves acetylcholinesterase inhibitors including Donepezil or Aricept, Rivastigmine, and Galantamine. Acetylcholinesterase inhibitors are used to increase the availability of acetylcholine and enhance cholinergic neurotransmission due to the degeneration of cholinergic neurons in AD (Blennow et al., 2006). Clinical trials have shown that these drugs can improve cognition by a mean of 1.4 points on the Mini-Mental State Examination (MMSE), although gastrointestinal side effects such as diarrhoea, nausea and vomiting are common (Blennow et al., 2006). A systematic review published in 2007 concluded that

cholinesterase inhibitors were not associated with any delay in the onset of AD when used by older adults with MCI (Raschetti, Albanese, Vanacore, & Maggini, 2007).

The second type of symptomatic treatment is a drug called Memantine, an N-methyl-D aspartate receptor antagonist. Memantine acts by protecting neurons from glutamate mediated excitotoxicity, and appears to be well tolerated with few adverse effects (Blennow et al., 2006). Clinical trials have shown that Memantine provides positive effects on both behavioural and cognitive symptoms in older adults with moderate to severe forms of AD, but not in early stages or milder forms. Benzodiazepines, anti-psychotic and anticonvulsant drugs are also used to help with anxiety, agitation and psychosis both in the early and late stages of dementia (Kozman, Wattis, & Curran, 2006).

Drugs which hold hope in modifying the pathology behind the disease have so far been tested using 'knockout mice' and are still in the early stages of development. These drugs have been developed to help alter the pathology of AD with secretase modulators to reduce amyloid *β* concentration in the brain, with amyloid *β* antibodies to reduce deposition, with amyloid *β* fibrillation inhibitors to reduce amyloid *β* plaque changes and with anti-tau drugs to reduce tau phosphorylation – all so far with very limited results (Blennow et al., 2006). Anti-inflammatory medications including both steroidal, such as prednisone, and nonsteroidal anti-inflammatory drugs (NSAID), such as the COX-2 inhibitor celecoxib, have been tested in clinical trials with AD but with disappointing results. Anti-inflammatory drugs are used to reduce excess or prolonged inflammation within the brain, which is thought to worsen neuronal damage and loss through pathological mechanisms including excitotoxicity and oxidative stress (In'T Veld, Launer, Breteler, Stricker, & Hofman, 2002; Crupi, Marino, & Cuzzocrea, 2013). The large Rotterdam study involving almost 7,000 older adults over 55 years of age looked at the relative risk of AD concerning NSAID use (In'T

Veld et al., 2001). The study found that long-term (24 months or longer) use of NSAIDs, but not aspirin use, was associated with a reduction in risk for AD but not VD. The authors suggest that there may be a critical period approximately two years before the onset of dementia where anti-inflammatory agents may be helpful, but less so after the symptomatic threshold has been passed. Cholesterol-lowering medications, specifically statins, have shown some promise. The use of statins is based on the associations drawn between high cholesterol levels, cardiovascular disease and vascular dementia, as well as the link between cholesterol and amyloid *β* production (Launer et al., 2002).

1.6 Summary

Whether or not a single risk factor dictates the development of MCI and AD pathology, whether vascular and metabolic processes cause the pathology to pass a 'dementia threshold', and exactly how genetics and environmental, lifestyle and psychosocial factors interact to contribute to the progression of the disease is unknown (Blennow et al., 2006). What is known for sure though, is that AD is a progressive, insidious, age-related disease and "there is an urgent need to find prevention approaches that are safe, effective and affordable" (Cole & Frautschy, 2010, p. 869). Given that there are limited treatment options available, prevention strategies should start well before or at least at the preclinical phase of cognitive decline such as MCI. In 2009, Petersen et al. stated in reference to MCI and AD that "if a disease modifying therapy or effective lifestyle intervention were available, one would want to intervene as soon as possible" (p. 1447). Identifying and understanding the negative and positive risk factors for cognitive decline and dementia, and then conducting RCTs in older adults with MCI could be the best way forward in reducing the incidence of dementia.

CHAPTER 2

RISK FACTORS FOR COGNITIVE DECLINE

Chapter 2 will review the major risk factors associated with cognitive decline in older adults in order to understand how important diet and nutrition are in preventing cognitive decline and dementia.

2.1 Risk factors

The risk factors associated with cognitive decline, specifically with MCI and AD, can be divided into three categories; those that are non-modifiable such as age and genetics, those that are modifiable such as smoking and physical inactivity, and those that are protective such as diet. Risk factors are also classified as negative (increases risk) and positive or protective (reduces risk). Here 'positive' and 'protective' both refer to factors which reduce risk, whereas in the abovementioned, 'protective' refers to risk factors which currently have very little (but increasingly growing evidence) to support their modifying effect. Much of the evidence for these risk factors comes from prospective, population-based, epidemiological and longitudinal studies, as well as systematic reviews and meta-analyses, although RCTs have now provided support for several of these risk factors.

2.2 Non-modifiable risk factors

Age is the single biggest non-modifiable risk factor for cognitive decline, MCI and late-onset AD (Dubois et al., 2016). The prevalence of dementia is approximately 1% in older adults between 60 and 64 years of age, whereas it is between 24 to 33% in older adults over the age of 85 (Blennow et al., 2006). The incidence of AD doubles in rate with every five years of life (Plassman et al., 2010). Age is also a significant risk factor for MCI, with prevalence rates for MCI of 6.7% for 60 to 64 years olds, 8.4% for 65 to 69 year olds, 10.1% for 70 to 74 year olds, 14.8% for 75 to 79 year olds and 25.2% for 80 to 84 year olds (Petersen et al., 2018). The number of older adults with dementia will continue to increase with the world's ageing population.

The second non-modifiable risk factor for cognitive decline, particularly for AD, is genetics. Mutations in the genes for amyloid β precursor proteins, presenilin 1 and presenilin 2 account for approximately 40% of early-onset AD, whereas a variant of the Apolipoprotein E (APOE) gene, APOE ε4, has been identified as the primary genetic risk factor for sporadic late-onset AD (Farrer et al., 1997; Puglielli, Tanzi, & Kovacs, 2003; Coon et al., 2007; Barberger-Gateau et al., 2011). The APOE gene is a glycoprotein, a protein attached to a sugar molecule, made up of 299 amino acids on chromosome 19 (Kim, Basak, & Holtzman, 2009). The APOE gene is expressed mainly in the liver and the brain, mostly in astrocytes and microglia, and to a lesser extent in neurons (Kim et al., 2009). One of the main roles of APOE is as a ligand or a binding molecule to receptors on lipoproteins. Lipoproteins are protein complexes that allow the body to transport hydrophilic substances, such as fats and lipids, within water-based fluids like blood. There are five main types of lipoproteins – high-density lipoproteins (HDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) and ultra-low-density

lipoproteins (ULDL) (Mora et al., 2007). HDL is often referred to as a 'good' lipoprotein because it transports fats and cholesterol from the cells back to the liver, whereas LDL is 'bad' as it transports fats and cholesterol from the liver to cells, and has been implicated in depositing fats and cholesterol within atherosclerosis plaques (Mora et al., 2007).

One of the roles of APOE is to help transport and clear LDL, VLDL and ULDL from circulation by acting via receptors on the liver and peripheral cells (Thakkinstian, Bowe, McEvoy, Smith, & Attia, 2006). Within the brain, APOE is the main carrier of cholesterol to neurons, and plays a role in stabilising the structure of lipoproteins as well as repairing and maintaining neuronal cell membranes (Puglielli et al., 2003; Thakkinstian et al., 2006). There are three alleles or variants of the APOE gene labelled as APOE ε2, APOE ε3 and APOE ε4. Humans carry two APOE alleles; one allele has been passed on from each parent. Individuals can be homozygous (carry two of the same alleles) or heterozygous (carry two different alleles) for the APOE gene, resulting in six variants or genotypes – ε2/ε2, ε2/ε3, ε2/ε4, ε3/ ε3, ε3/ε4 and ε4/ε4 (Barberger-Gateau et al., 2011). The most common variant is ε3, which is carried by approximately 75% of Caucasians, followed by ε4 which is carried by approximately 15% of Caucasians and ε2 by approximately 10% (St George-Hyslop, 2000). Approximately 0.9% of Caucasians carry ε2/ε2, 12% carry ε2/ε3, 60.6% carry ε3/ε3, 2.1% carry ε2/ε4, 22.4% carry ε3/ε4 and 2% carry ε4/ε4 (Alzheimer Research Forum, 2010). Research has shown variations in the prevalence of APOE allele carriers within different ethnic groups. For example, a meta-analysis by Thakkinstian et al. (2006) found no difference between Caucasians and Asians in the prevalence of ε2 carriers, but a higher prevalence of ε4 carriers in Caucasians than in Asian populations.

Farrer et al. (1997) computed odds ratios (OR) for risk of AD in Caucasians according to the six APOE genotypes from 5930 AD patients and 8607 controls. The results showed a

significant increase in risk for AD in the three ε4 genotypes, ε2/ε4 (OR = 2.6), ε3/ε4 (OR = 3.2) and ε4/ε4 (OR = 14.9), as well as a significant decrease in risk for ε2/ε2 (OR = 0.6) and ε2/ε3 (OR = 0.6) compared to ε3/ε3 carriers. Results from this study and several others indicate that carrying one ε4 allele results in a 2 to 3-fold increased risk for developing AD, whereas carrying two ε4 allele results in a 12 to 15-fold increased risk in developing AD compared to carrying two ε3 alleles (Coon et al., 2007; Barberger-Gateau et al., 2011). The relationship between the APOE ε4 allele and the onset of AD appears to be dose-dependent, in that homozygous ε4 carriers (ε4/ ε4) tend to develop AD before 70 years of age, whereas heterozygous carriers develop AD later than 70 (St George-Hyslop, 2000). Carrying ε2 has been shown to have a protective role on cognition, and if carriers of ε2 develop AD, it tends to be after 90 years of age. For example, in 85 older adults over the age of 90, ε2 carriers were found to be less likely to be diagnosed with dementia, but along with ε4 carriers, to be more likely to meet the neuro-pathological criteria for AD than ε3 carriers (Berlau, Corrada, Head, & Kawas, 2009). These authors suggest that ε2 may contribute to maintaining healthy cognition in the oldest old independently of AD pathology.

Several mechanisms have been proposed to explain the roles that APOE and the ε2, ε3 and ε4 alleles play within the brain. Research shows that plasma APOE levels decrease as a function of the progression from normal cognition through to MCI and AD (Soares et al. 2012) and that ε4 carriers have lower APOE levels in the blood, brain and CSF than ε2 and ε3 (Poirier et al., 2014). APOE is the main carrier of cholesterol within the brain and plays an important role in cholesterol homeostasis. A large study of 1089 men aged between 65 and 84 found that APOE status was associated with serum total and LDL cholesterol levels, specifically that the genotypes ε2/ε2, ε2/ε3, ε2/ε4, ε3/ ε3, ε3/ε4 and ε4/ε4 were associated with respectively increasing serum cholesterol levels (Giltay et al., 2009).

Research also indicates that ε4 binds more readily to amyloid *β* peptide than ε2, that amyloid *β* deposition may start earlier in ε4 carriers than non-ε4 carriers and that ε2 may be more effective at clearing soluble amyloid than ε4 (St George-Hyslop, 2000; Kim et al., 2009). Berlau et al. (2009) suggested that ε2 might provide a protective effect by maintaining synaptic integrity via higher levels of synaptophysin in the frontal cortex and by modifying the formation of tangles based on the finding that ε2 carriers had increased plaque but not tangle pathology. Drzezga et al. (2009) found that ε4 carriers had significantly higher amyloid *β* deposits particularly in the left temporal lobe, but also in both hemispheres in the temporoparietal and pre-frontal regions, than non-ε4 carriers.

A large meta-analysis that included 77 studies involving 40,942 cognitively healthy older adults found that ε4 carriers performed significantly worse on measures of episodic memory, global cognitive ability, executive functioning, and perceptual speed than non-ε4 carriers, with homozygous ε4 carriers performing significantly worse than heterozygous ε4 carriers on episodic memory when compared to homozygous ε3 carriers (Wisdom, Callahan, & Hawkins, 2011). Wang, Hong, Lin, Liu, and Chen (2011) found that ε4 carriers had a higher MCI to AD conversion rate than non-ε4 carriers, and that normal ageing subjects who were ε4 carriers also progressed to AD faster than non-ε4 carriers. Another study found an interaction between age and ε4 carrier status (Filippini et al., 2011). Older (mid-sixties) ε4 carriers were found to have decreased activation in the frontal and temporal lobes, cerebellum and hippocampi as well as reduced cerebral blood flow whereas young (mid-twenties) ε4 carriers showed increased brain activity. A much earlier study by Tierney et al. (1996) found that ε4 carrier status was only a reliable indicator of developing AD when performance on a memory test (e.g., Rey Auditory Verbal Learning Test (RAVLT)) was included in the predictive model. There is no doubt that age is the biggest risk factor for AD,

and although APOE ε4 has been identified as the main genetic risk factor for late-onset AD, it seems that it is neither required nor sufficient on its own to cause AD (Tanzi & Bertram, 2001; Puglielli et al., 2003).

2.3 Modifiable risk factors

Researchers have identified several modifiable risk factors that have 'sufficient', 'consistent' or at least 'moderate to strong' evidence for increasing the risk of cognitive decline and dementia. These factors include diabetes, midlife obesity and hypertension, smoking, depression, low educational attainment or cognitive inactivity, physical inactivity and traumatic brain injury (Plassman et al., 2010; Barnes & Yaffe, 2011; Deckers et al., 2015; Baumgart et al., 2015). In addition, several other risk factors such as sleep disturbances, alcohol intake and cholesterol levels have also been linked to an increased risk of dementia, although low to moderate alcohol consumption is also associated with a decreased risk (Anstey, Mack, & Cherbuin, 2009). These modifiable risk factors work directly or indirectly and several show significant overlap in terms of the mechanisms by which they increase the risk of dementia.

The evidence linking diabetes with an increased risk for dementia and lower cognitive performance is considered to be very strong (Baumgart et al., 2015). For example, a meta-analysis of longitudinal studies found that diabetes was a risk factor for MCI, AD and VD, and those with diabetes have more than double the risk for developing VD (Cheng, Huang, Deng, & Wang, 2012). A meta-analysis of prospective observational studies found that older adults with a history of diabetes compared to those without showed a 73% increased risk of all types of dementia, a 56% increased risk for AD alone and a 127% increased risk for VD. A third meta-analysis found that older adults with MCI and diabetes

were more likely to convert to dementia than those without diabetes (Cooper, Sommerlad, Lyketsos, & Livingston, 2015). The mechanisms by which diabetes increases dementia risk is via vascular pathways (diabetes increases the risk for strokes and infarctions), and directly through glucose mediated toxicity and reduced insulin receptor activation (Cooper et al., 2015).

A strong link has also been found between midlife obesity and an increased risk for dementia (Baumgart et al., 2015). A meta-analysis of longitudinal, epidemiological studies found that obesity significantly increased the risk for AD (Profenno, Porsteinsson, & Faraone, 2010). Another meta-analysis found that midlife underweight BMI, overweight BMI and obese BMI were all associated with increased risk of dementia, implying a U-shaped relationship where both being underweight and overweight increase the risk of dementia (Beydoun, Beydoun, & Wang, 2008). Adiposity or being severely overweight is thought to affect the brain through different biological mechanisms including by altering energy metabolism and by increasing inflammation via cytokines (Gustafson, 2006). Calorie restricting or fasting programs have been shown to improve cognition in healthy older adults, which may be due to improved insulin sensitivity and reduced inflammatory activity (Witte, Fobker, Gellner, Knecht, & Flöel, 2009). A meta-analysis by Loef and Walach (2013) also found a relationship between midlife obesity and dementia risk, as well as a lower risk of dementia in those who were overweight compared to those with a normal weight in later life. The authors suggest this result may be due to low BMI being a marker for dementia, due to dementia-related brain atrophy affecting brain regions implicated in appetite control and energy balance (Loef & Walach, 2013).

The evidence linking midlife hypertension with dementia is strong, yet inconsistencies have been found (Barnes & Yaffe, 2011; Baumgart et al., 2015). A systematic

review of longitudinal and cross-sectional prospective studies found that hypertension significantly increased the risk for VD (Sharp, Aarsland, Day, Sønnesyn, & Ballard, 2011), and a large (11,151 adults between 46 and 70 years) population-based prospective study found that diabetes, hypertension and smoking were all strongly associated with a higher risk of hospitalisation with dementia, particularly in middle-aged participants (Alonso et al., 2009). Two meta-analyses found no association between hypertension and risk of AD (Guan et al., 2011; Power et al., 2011), although some have suggested that these results may be due to combining mid and late life hypertension (Qiu, 2012). The use of hypertension medications for reducing the risk for cognitive decline has also been extensively studied, but again, with mixed results (Chang-Quan et al., 2011; Rouch et al., 2015). Long term or chronic high blood pressure leads to a thickening of the vessel walls, which narrows arteries resulting in infarctions (obstruction of the blood supply) including within the cerebral areas. Exactly why hypertension in later life is not associated with an increased risk for AD or VD (in fact has a protective effect against cognitive decline) whereas hypotension in late life is associated with an increased risk of dementia, is unclear, although it is likely due to the reduced regulatory capabilities of cerebral arteries (Kennelly, Lawlor, & Kenny, 2009).

There is strong evidence linking smoking with cognitive decline and dementia (Baumgart et al., 2015). A meta-analysis of prospective cohort studies found that current smokers had an increased risk for AD, VD and all-cause dementia, and that ceasing smoking decreased the risk to that of those who had never smoked (Zhong, Wang, Zhang, Guo, & Zhao, 2015). Another meta-analysis found that current smokers, compared to those who had never smoked, had a 40 to 80% increased risk of dementia and cognitive decline (Anstey, von Sanden, Salim, & O'Kearney, 2007). In a large cohort study, smoking in midlife was associated with risk of dementia in a dose dependent manner with heavy smokers

(more than two packs per day) with the greatest risk (Rusanen, Kivipelto, Quesenberry, Zhou, & Whitmer, 2011). Smoking is thought to affect brain health in several indirect ways via vascular, inflammatory, and degenerative (e.g., oxidative stress from the free radicals in cigarette smoke) processes (Collins, Sachs-Ericsson, Preacher, Sheffield, & Markides, 2009).

A Delphi consensus study rated depression as the most important risk factor for dementia, whereas a population-based report concluded that the link between depression and cognitive decline is unclear (Deckers et al., 2015; Baumgart et al., 2015). Two systematic reviews and meta-analyses found that older adults with a history of depression were more likely to have AD in later life (Ownby, Crocco, Acevedo, John, & Loewenstein, 2006), and that a history of depression was associated with a significant risk for all-cause dementia and AD, and was the highest for VD (Diniz, Butters, Albert, Dew, & Reynolds, 2013). Studies also indicate that high levels of depression increase the risk of MCI progressing to dementia (Modrego & Ferrández, 2004). It is still unclear whether depression is a symptom or early marker of dementia, whether depression is a risk factor for dementia or whether depression is triggered by a diagnosis of dementia, with some suggesting that depression is part of the cognitive decline continuum in specific subsets of older adults (Panza et al., 2010). Multiple factors contribute to the etiology of depression, from neurobiological factors such as gender (being female), dysregulation of the limbic, serotonergic and immune systems, to family and precipitating factors such as childhood adversity or trauma, high levels of stress, low sunlight, life events and transitions including retirement, along with personality and social factors such as neuroticism, pessimism and a lack of social support and loneliness (Carr & McNulty, 2016). Several hypotheses such as poor vascular health, neuronal damage (particularly in the frontostriatal circuit) and long-term inflammation have been proposed to explain the link between late-life depression and dementia (Panza et al., 2010).

There is strong evidence for the link between low educational attainment (the number of years spent in formal education) and risk for dementia (Baumgart et al., 2015). A large systematic review and meta-analysis involving 437,477 older adults found that those with a low education had a higher risk for dementia (Meng & D'Arcy, 2012), and another meta-analysis found low level of education to be an independent risk factor for AD (Caamaño-Isorna, Corral, Montes-Martínez, & Takkouche, 2006). A large study found education had a protective effect on the risk for developing AD in a dose-dependent manner, in that the protection increased with the number of years of education (Sando et al., 2008). This study also found carriers of the APOE ε4 allele had an increased odds ratio for developing AD, and that males had a lower risk for AD than females, which the authors suggest was possibly related to years of education. Analysis of 470 Catholic sisters from a large Nun study found that age, educational attainment and carrying the APOE ε4 allele influenced the odds for developing dementia, and that all three were significant predictors for the transition from normal cognition to mild cognitive impairments (Tyas et al., 2007).

The construct or hypothesis of cognitive reserve has been proposed to explain why higher educational attainment might reduce the risk of dementia (Stern, 2012). Cognitive reserve is the ability to use compensation mechanisms, to draw upon multiple regions of the brain or to utilise the regions of the brain that are working in a more efficient way, when other regions are affected by neuronal degeneration (Meng & D'Arcy, 2012). Cognitive reserve is used to describe a state of discrepancy found in the brain function of older adults, where in some older adults a greater level of disease pathology needs to occur before clinical symptoms appear (Stern, 2012). The capacity to develop compensatory mechanisms or neural scaffolding (Park & Reuter-Lorenz, 2009) has been shown to be strongly related to the level of participation in cognitive activities. A large study (2,498 cognitively healthy older

adults and 809 older adults with MCI) found that older adults with MCI showed less employment of these compensatory mechanisms and that greater participation in cognitive activities (reading books, using a computer, operating a video or DVD player, using a bus or a train and management of finances) was associated with lower odd ratios of MCI (Doi et al., 2013). A systematic review of seven RCTs found that cognitive training produced a strong and persistent protective effect on cognitive performance in cognitively healthy older adults (Valenzuela & Sachdev, 2009). A systematic review of 13 cohort observational studies found an association between participating in cognitive leisure activities and a reduced risk for developing AD (Stern & Munn, 2010). Together with education and cognitive activity, occupational attainment, physical exercise and life experiences have also been shown to increase cognitive reserve and neural scaffolding capacity.

Physical inactivity has been shown to be strongly linked to an increased risk of dementia and cognitive decline (Baumgart et al., 2015). A systematic review of 16 prospective studies found that physical activity was inversely associated with risk for dementia, and overall reduced risk for dementia by 28% and AD by 45% (Hamer & Chida, 2009). A meta-analytic review of 29 RCTs undertaken by Smith et al. (2010) found the aerobic exercise training was associated with 'modest' improvements in cognitive performance particularly in attention, processing speed, executive functioning and memory, with moderator analysis finding that combining aerobic exercise with strength training improved cognitive performance to an even greater extent. Smith et al. also found preliminary evidence to suggest that participants with MCI showed a greater improvement in memory from exercise than participants who were non-cognitively impaired.

A RCT involving 120 older adults found that moderate-intensity aerobic exercise three days per week resulted in improved spatial memory, and increased the size of the

anterior hippocampus by 2%, which is equivalent to an age-related loss in volume of between 1 to 2 years (Erickson et al., 2011). The RCT also found that the increase in hippocampal size was associated with higher serum levels of brain-derived neurotrophic factor (BDNF). The mechanisms by which physical activity influences cognitive decline is via vascular factors such as lowering blood pressure and improving cerebral circulation (blood flow and oxygen supply), by enhancing growth factors such as BDNF and by lowering anti-inflammatory markers (Hamer & Chida, 2009). It is still unclear what type and intensity of exercise are required, and how long the physical activity needs to be in order to maximise the protective effects of exercise (Baumgart et al., 2015).

There is now solid evidence that moderate to severe traumatic head or brain injury (TBI) increases the risk for dementia (Baumgart et al., 2015). A large study involving 188,764 US veterans over 55 years of age found that TBI was associated with a 60% increased risk for developing dementia (Barnes et al., 2014). Another study involving 2,552 retired professional US football players found that those players who had sustained three or more concussions had a 5-fold prevalence of MCI and 3-fold prevalence in memory problems (Guskiewicz et al., 2005). Another military-based study with 811,622 Swedish men found a strong association between risk for early-onset dementia of non-AD forms and TBIs of different levels of severity (Nordström, Michaëlsson, Gustafson, & Nordström, 2014). Explanations for why TBI increases risk for dementia include that a head injury might trigger the pathological development of tangles and plaques, white matter degradation, general neuronal loss and cerebral atrophy and chronic neuro-inflammation. Other factors such as carrying the APOE ε4 allele also seem important, as the APOE gene plays an important role in the response of the brain to injury (Guskiewicz et al., 2005; Blennow et al., 2006; Smith, Johnson, & Stewart, 2013).

Several cohort and observational studies have indicated that sleep disturbances increase the risk of cognitive decline (Baumgart et al., 2015). A prospective study found that women with sleep-disordered breathing had an increased risk of developing cognitive impairment (Yaffe et al., 2011). A five-year follow-up population-based study found that sleep apnoea participants had a higher risk of developing dementia (Chang et al., 2013), and a large population-based study with 30,038 older adults found that sleep disturbances were associated with an increased risk of developing AD and dementia (Sterniczuk, Theou, Rusak, & Rockwood, 2013). A study involving 101 older adults with AD found that poor subjective sleep quality, sleep problems and daytime sleepiness were associated with greater AD pathology such as CSF plaques (Sprecher et al., 2017). Sleep affects cognition by disrupting long-term memory consolidation, by way of hypoxia and reducing cerebral blood flow and grey matter (Yaffe et al., 2011). Yet again, it is unclear whether sleep disturbances are a cause of or are a precursor to dementia (Baumgart et al., 2015).

Alcohol consumption and cholesterol levels are both negative and positive risk factors for dementia, in that low to moderate levels of alcohol show a protective effect and yet high levels are associated with brain damage, and cholesterol levels can be a positive or negative risk factor depending on the type of cholesterol measured. For example a meta-analysis of 15 prospective studies found that low to moderate alcohol consumption was associated with a 25 to 28% reduction in risk for dementia compared to those who did not drink, although several studies have found this reduction in risk for wine only (Anstey et al., 2009; Luchsinger Tang, Siddiqui, Shea, & Mayeux, 2004). A systematic review of 18 prospective studies found that high midlife total serum cholesterol increases the risk of dementia (Anstey, Lipnicki, & Low, 2008) whereas in a large population-based cohort late-life total serum cholesterol was not associated with dementia (Tan et al., 2003) and a

prospective cohort study found that high levels of HDL were associated with a decreased risk of AD (Reitz et al., 2010). Low to moderate alcohol consumption may reduce dementia risk by increasing HDL levels which lowers cardiovascular risk, increasing cerebral blood flow, decreasing coagulation and reducing inflammation, whereas heavy consumption is a risk factor for most stroke subtypes and causes alcohol-related brain damage such as cortical volume deficits, cerebral shrinkage, neurotoxicity, neuro-inflammation and changes in neurotransmitter production (Anstey et al., 2009; Zahr, Kaufman, & Harper, 2011).

While the evidence for these modifiable risk factors is strong, all epidemiological evidence suffers from the inability to infer causality. It is difficult to be sure, for example, whether low physical activity levels result in accelerated cognitive decline or a cognitive deficit causes a reduction in physical activity. What is more apparent, is that these risk factors have very similar mechanisms, in that most risk factors affect cognition via vascular, inflammatory or insulin receptor pathways. Educational attainment and cognitive activity is the exception, where it is thought to protect via an increased brain reserve capacity; although again it is uncertain as to whether low levels of cognitive engagement contribute to the development of MCI and AD or whether those with MCI are less likely to engage in cognitive activities due to their declining cognition. These modifiable risk factors are also highly interrelated; for example, obesity and physical inactivity are related to socioeconomic status, which is related to education and occupation (Cerin & Leslie, 2008).

2.4 Protective factors

Researchers have identified several other modifiable risk factors which have been classified as having ‘very limited’ but increasing evidence for reducing the risk of cognitive

decline and dementia. The two risk factors that will be discussed here are social connection or engagement, and the effects of diet and nutrition.

Several studies have shown that social connection or social engagement, including having regular social activities, larger social networks, and a history of social contact, are associated with better cognitive function and reduced risk of cognitive decline (Baumgart et al., 2015). A prospective study of 2,249 women over 78 years of age found that women with larger social networks had a lower risk for dementia (Crooks, Lubben, women, Little & Chiu, 2008) and another study with 1,138 older adults without dementia found more social activity was associated with less cognitive decline during average follow-up of 5.2 years (James, Wilson, Barnes, & Bennett, 2011). A study involving 6,102 older adults found that greater social networks and social engagement was associated with reduced cognitive decline (Barnes, De Leon, Wilson, Bienias, & Evans, 2004). Another study assessing the effects of physical, social and mental components of leisure activities in 2,368 older adults from Sweden found that the social element of activities was just as beneficial as the physical and mental components for reducing the risk of dementia (Karp et al., 2006). In contrast, a coordinated analysis of four longitudinal studies did not find an association between social activity and cognitive functioning (Brown et al., 2012). Social engagement is thought to provide a protective effect on cognition by reducing stress, depression and loneliness, by enhancing resilience and by building scaffolding or cognitive reserve due to the complexities and challenges involved in communication (Barnes et al., 2004; Crooks et al., 2008; James et al., 2011).

The evidence for the direct effects of diet and nutrition on reducing the risk for cognitive decline and dementia is considered to be inconsistent and limited (Baumgart et al., 2015), although a healthy diet was rated as the sixth most important risk factor for dementia

by a Delphi consensus study (Deckers et al., 2015). Research investigating the link between food, diet and cognition can be divided into four areas – micronutrients (a substance needed in a small quantity, but which is necessary for normal metabolism and growth) such as vitamin E and flavonoids, macronutrients (a type of nutrient required in large amounts) such as fats and carbohydrates, specific foods or food groups such as fish or fruit and vegetables, and diets or whole dietary patterns such as the Mediterranean diet. Several micronutrients have been studied in terms of reducing the risk for dementia (by reducing atrophy in MCI and improving cognition) including vitamin E, D and B12, folate (vitamin B9) and homocysteine (a by-product of amino acid metabolism) but with inconsistencies and limited support (e.g., Petersen et al., 2005; Ford & Almeida, 2012; Douaud et al., 2013).

Research indicates that the damage caused by AD pathology is associated with damage from oxidative stress, a term used to describe the damage caused to cells from free radicals as a result of a series of chain reactions involving oxygen (Launer et al., 2002). Free radical agents or anti-oxidants interact with free radicals (by donating or accepting electrons) to interrupt and terminate this chain reaction (Opara & Rockway, 2006). There are many different types of anti-oxidants found in food from vitamins and minerals such as the vitamin C in citrus fruit and selenium in Brazil nuts, certain types of acids (e.g., benzoic and cinnamic acid) found in onions and coffee, and pigments such as carotenes and flavonoids. Carotenes and flavonoids are found in plants as brightly coloured pigments and have been shown to have potent anti-oxidant and anti-inflammatory effects (Gillette-Guyonnet et al., 2007). Plants with particularly high levels of carotenes and flavonoids include orange and yellow fruits and vegetables such as carrots and mangoes, berries especially blueberries and blackcurrants, black and green tea from the camellia plant, dark chocolate from the cacao plant, and wine, especially when produced with the skins of red

grapes (Opara & Rockway, 2006). The Rotterdam study found the use of anti-oxidant supplements such as beta-carotene and flavonoids as well as intake of vitamin C and E was associated with a lower risk for AD (Engelhart et al., 2002).

Several macronutrients and food groups have been linked to both improvements and impairments in cognition through the lifespan. These include fats or fatty acids, particularly *n*-3 PUFAs found in oily fish and the saturated fatty acids found in meat and dairy, also simple and complex carbohydrates (the link between cognition and insulin insensitivity) as well as fruit and vegetables and water. Along with fish consumption, fruit and vegetable consumption has been shown to play a protective role in reducing the risk of dementia. A large cohort study involving 8,085 healthy older adults over 65 years of age found that daily consumption of fruits and vegetables was associated with a decreased risk for dementia (Barberger-Gateau et al., 2007), and a systematic review of nine cohort studies found increased vegetable, but not fruit intake, was associated with a lower risk of dementia and a slower rate of decline (Loef & Walach, 2012). Reasons why vegetable consumption may be more protective than fruit could be due to the lower sugar and higher fibre (which reduces total serum cholesterol levels) content along with the higher level of flavonoids and micronutrients.

Research investigating the protective effects of the Mediterranean diet have been very promising. The Mediterranean diet is characterised by a diet high in fruit and vegetables, whole grains, legumes and nuts, moderate in dairy, low in red meat, with fish and olive oil as sources of protein and fat, and with a moderate level of wine consumption. Evidence suggests that people who maintain diets like those which proliferate in the Mediterranean (Italy, Spain and Greece) as well as in North East Asia (without olive oil, instead soya bean oil and fermented soya bean products such as tofu, tempeh and miso)

have lower rates of chronic disease, coronary health disease, cancer, neuro-degenerative conditions such as Parkinson's disease and AD, a reduced risk of progressing from MCI to AD as well as a higher life expectancy (Willett et al., 1995; Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003; Knoops et al., 2004; Scarmeas, Stern, Tang, Mayeux, & Luchsinger, 2006; Sofi, Cesari, Abbate, Gensini, & Casini, 2008; Estruch et al., 2013; Singh et al., 2014). Possible mechanisms underlying the protective effects of the Mediterranean diet include via vascular, oxidative and anti-inflammatory pathways, quite possibly from the interaction of components and nutrients of the diet (Frisardi et al., 2010). The Mediterranean diet is also characterised by a minimal level of food processing, the use of fresh and seasonal fruits and vegetables which further increases the fibre, anti-oxidant and micronutrient content (Willett et al., 1995). Recently a large study involving 2,796 adults in France aged between 45 and 60 years found that a pro-inflammatory diet was associated with a decreased likelihood of healthy ageing, indicating that the anti-inflammatory effect of a diet maybe the significant factor (Assmann et al., 2018).

Support for a diet, social and lifestyle interaction comes from demographic work into regions of the world coined 'Blue zones' (Sardinia in Italy, Okinawa in Japan, Loma Linda in California, the Nicoya Peninsula in Costa Rica and Icaria in Greece) with particularly high rates of longevity. Researchers found that the people who lived in these areas shared several characteristics including a moderate calorie, plant-based (semi-vegetarian with high legume consumption) diet along with regular physical activity, moderate alcohol consumption, strong social and family connections, a life purpose and an emphasis on stress reduction (Poulain, Herm, & Pes, 2013). A recent 3-year RCT called the Multi-domain Alzheimer Preventive Trial aimed to investigate the effects of a multi-domain intervention combining cognitive training, physical activity and *n*-3 PUFAs in 1,680 non-demented older

adults with memory complaints over 70 years of age. The trial did not find a significant effect on cognitive decline, except in executive functioning in the *n*-3 PUFA group with low baseline levels of *n*-3 PUFAs (Andrieu et al., 2017; Hooper et al., 2017). An earlier 2-year multi-domain (diet, exercise, cognitive training and vascular risk monitoring) RCT with 1,260 older adults between 60 and 77 years of age did find a significant intervention effect on overall cognition (composite score from 14 tests), secondary cognition (executive functioning and processing speed), but not memory (Ngandu et al., 2015). The authors note that considering the multifactorial nature of cognitive decline and dementia, "a one-size-fits-all solution is unlikely to be found" and that "tailoring interventions to risk profiles and different geographical and cultural settings should be the focus of future research" (Kivipelto, Mangialasche, & Ngandu, 2017, pg. 339).

2.5 Summary

Given that most risk factors for cognitive decline are interrelated and cumulative, the best prevention approaches are likely to be multifactorial or 'pleiotropic' with multiple benefits such as a healthy lifestyle with regular exercise and a balanced diet (Baumgart et al., 2015). In terms of diet, components of the Mediterranean diet such as the consumption of oily fish and vegetables alongside physical exercise and cognitive training shows promise and deserves further exploration. Exactly how and why the typical Western-style diet has veered so far away from the Mediterranean or 'blue zone' way of eating is unclear, although the large scale production and advertising of food high in refined sugar and certain types of fats is a likely culprit. The type of nutrient that has received the most attention in reducing cognitive decline are the *n*-3 PUFAs found in oily fish, and are the focus of Chapter 3 and 5, which will discuss the mechanisms and evidence for the use of these fatty acids in improving

cognition in older adults. Evidence from RCTs provides the best support for prevention strategies, although variation from person to person in terms of the latent time period between the beginning of pathological mechanisms and the onset of clinical symptoms confounds results. Important considerations for prevention trials are an adequate sample size, an appropriate age range, including specific risk profiles, the strength of the intervention as well as choosing sensitive cognitive measures to detect meaningful cognitive changes.

CHAPTER 3

OMEGA-3 FATTY ACIDS AND COGNITION

This chapter will examine the chemistry, metabolism and pharmacological effects of omega-3 polyunsaturated fatty acids (*n*-3 PUFA) before reviewing the epidemiological evidence and mechanisms for the cognitive effects of *n*-3 PUFAs in older adults.

3.1 Fatty acids

Fats or fatty acids have received a bad name, mainly because of the high energy density (37 kilojoules per gram compared to 17 kilojoules per gram for protein and carbohydrates), but also because of the link between certain types of fatty acids such as saturated fatty acids and trans-fatty acids with coronary heart disease and cholesterol levels (Capra et al., 2006; Kromhout et al., 1995). However, it was not until quite recently that the detrimental effects of a low-fat diet, particularly a diet low in *n*-3 PUFAs on growth and cognition have become known. Nutritional research shows that most older adults who eat a Western-style diet have insufficient dietary intake and blood levels of *n*-3 PUFAs, possibly due to increased acute and chronic illnesses, impaired digestion, changes in taste and smell as well as the difficulties associated with living, shopping and eating alone (Bourre & Paquotte, 2008; Cole & Frautschy, 2010). Symptoms of a deficiency in *n*-3 PUFAs include fatigue, visual disturbances, scaly skin and reduced growth in children (Capra et al., 2006).

Whether the low intake of *n*-3 PUFAs in older adults can also explain the high incidence of conditions such as AD is unclear.

3.2. Chemistry and metabolism of fatty acids

Most fatty acids in the human body are in the form of triglycerides, phospholipids and cholesterol (Capra et al., 2006). Triglycerides are found in the blood, adipose tissue and in sebum secreted by sebaceous glands in the skin, whereas most phospholipids are found within cell membranes (Yehuda, Rabinovitz, Carasso & Mostofsky, 2002). A phospholipid is made up of two fatty acids joined by a phosphate group head. The phosphate head end is hydrophilic or water attracting, whereas the tail end is hydrophobic or water repelling. Two phospholipids join up tail to tail (as both tails are water repelling) to form a phospholipid bilayer with the phosphate heads on the inside and outside of the cell membrane. This important structure provides the cell membrane with a barrier to control the movement of substances in and out of the cell (Yehuda et al., 2002).

A fatty acid is made up of a carbon atom chain with a methyl end and a carboxyl or acid end. The number of carbon atoms in a fatty acid can be anywhere between 3 and 38, or more. Fatty acids can be further divided into saturated, monounsaturated or polyunsaturated based on whether the carbon chain contains a double bond or not (White, 2009). If a fatty acid contains no double bonds (i.e., contains only single bonds) between the carbons, it is called a saturated fatty acid (SFA). If the fatty acid contains one double bond in the carbon chain it is called a monounsaturated fatty acid (MUFA), and if it contains two or more double bonds it is called a polyunsaturated fatty acid (PUFA) (White, 2009). The three fatty acids that make up a triglyceride can be a combination of any of these three fatty acids,

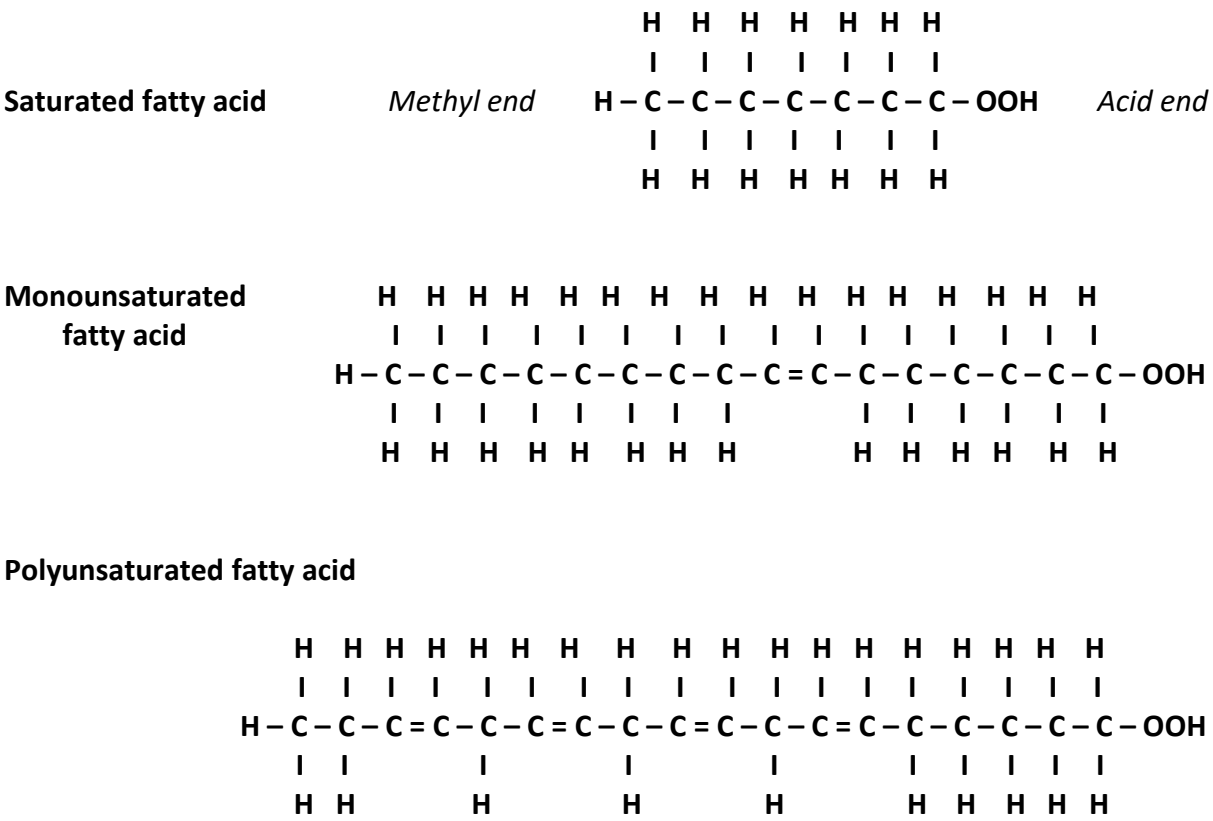

Figure 1. Fatty acid carbon chains derived from White (2009)
Note. C = Carbon, H = Hydrogen, O = Oxygen

for example, one SFA and two PUFAs. PUFAs typically contain between 2 to 6 double bonds and are referred to as omega-3, omega-6 (*n*-6 PUFA) or omega-9 (*n*-9 PUFA) polyunsaturated fatty acids depending on whether the first double bond is at the 3rd, 6th or 9th carbon atom. The double bond in a MUFA is usually on the 7th or 9th carbon. In this system, the number of the first carbon in the chain is determined by counting the carbon atoms from the methyl end. See Figure 1.

SFAs are found in animal meats and various types of milks, palm kernel and coconut, MUFAs in olives, avocados, nuts and plant oils, and PUFAs in seeds, meat and oily fish (Capra et al., 2006). See Table 2 for the most common fatty acids, along with the lipid number and food sources for each one. The lipid number indicates how many carbon atoms are in the

Table 2. The most common types of fatty acids derived from White (2009)

FA	Common name	Lipid number	Food sources
S	Butyric acid	C4	Animal milk – butter, cheese
S	Caprylic acid	C8	Animal milk, coconut, palm kernel oil
S	Lauric acid	C12	Animal milk, coconut, palm kernel oil
S	Palmitic acid	C16	Dairy, meat, palm oil, cocoa butter
M	Palmitoleic acid	C16:1*n*-7	Macadamia, coconut, palm oil
S	Stearic acid	C18	Meat, cocoa butter, shea butter
M	Oleic acid	C18:1*n*-9	Olive oil, avocado, pecans, canola oil
P	Linoleic acid	C18:2*n*-6	Safflower, sunflower oil, corn oil
P	Alpha-linolenic acid	C18:3*n*-3	Flax/chia seeds, leafy green vegetables
P	Stearidonic acid	C18:4*n*-3	Hemp, blackcurrant seeds, spirilina
M	Eicosenoic acid	C20:1*n*-9	Jojoba oil
P	Dihomogamma linolenic	C20:3*n*-6	Evening primrose oil, borage oil
P	Mead acid	C20:3*n*-9	Cartilage
P	Arachidonic acid	C20:4*n*-6	Meat, eggs, dairy
P	Eicosapentaenoic acid	C20:5*n*-3	Oily fish, seaweed/algae, eggs
P	Docosapentaenoic acid	C22:5*n*-6	Oily fish, beef, seal, maternal milk
M	Nervonic acid	C24:1*n*-9	Oily fish, flaxseeds, sesame seeds
P	Docosahexaenoic acid	C22:6*n*-3	Oily fish, maternal milk
P	Nisinic acid	C24:6*n*-3	Fish

Note. FA = Fatty acid: S = Saturated, M = Monounsaturated, P = Polyunsaturated

chain, how many double-bonds, if any, are in the carbon chain, and which carbon the first double bond is on. For example, a lipid number of 'C16' indicates that the fatty acid has 16 carbons in the carbon chain, with no double bonds and is therefore a SFA. A lipid number of 'C22:6*n*-3' indicates that the fatty acid has 22 carbons in the chain, with six double bonds with the first double bond on the 3rd carbon and is therefore an *n*-3 PUFA. PUFAs tend to be long-chained, that is contain at least 13 carbon atoms as opposed to SFAs which tend to be short chained and MUFAs which are often medium chained.

The properties and functions of a fatty acid largely depend on the number of carbons and the number of double bonds in the carbon chain (Bradbury, 2011). SFAs contain no double bonds and therefore are 'saturated' with hydrogen because the ratio of hydrogen to carbon is high (White, 2009). SFAs are solid at room temperature, carry no chemical

charge (are stable), are slow to react with other chemicals, and mostly provide energy, insulation and fat-soluble vitamin absorption. Long-chained SFAs, that is a fatty acid with more than 13 carbons, are found in cell membranes but because SFAs tend to aggregate, they lend a rigid structure to cell membranes. MUFAs contain one double bond which causes a bend or kink to form in the carbon chain (not shown in Figure 1). This kink is caused by the negative charge of the double bond, causing the fatty acids to repel each other, and therefore disperse as opposed to aggregate. This dispersing action means these fatty acids tend to be liquid at room temperature, and when found in cell membranes they lend more fluidity to the structure.

PUFAs contain multiple double bonds and are always liquid at room temperature. With every double bond the fatty acid has, the more kinks it has and the more dispersed it becomes. The human body uses PUFAs for hormone-like functions, to produce anti-inflammatory agents, to generate electrical currents (especially nerve impulses) and to create membranes that are very fluid which make it easier for substances to move in and out of cells. The human body will use long-chained PUFAs as an energy source, but only if other requirements have been met. Humans need a certain balance of the three types of fatty acids for optimal health. If there is insufficient PUFA in the diet, SFAs and MUFAs are used in replacement, but because these fatty acids do not have the same properties as PUFAs, the cell becomes less able to perform certain functions (Yehuda et al., 2002; Hulbert, 2003). Humans also need a certain ratio of *n*-3 PUFAs to *n*-6 PUFAs, ideally a ratio of 1:3, which is uncommon in Western-style diets due to the high consumption of foods deep fried in *n*-6 PUFA vegetable oils and the low consumption of oily fish, seaweed or algae and *n*-3 PUFA containing seeds (Yehuda et al., 2002). Western-style diets typically contain a ratio of 1:15 *n*-3 PUFAs to *n*-6 PUFAs, which is thought to contribute to the high rate of

inflammatory diseases, heart disease and neurological disorders in Western countries (Simopoulos, 2002; Denis, Potier, Vancassel, Heberden & Lavialle, 2013).

Humans are able to produce or synthesise SFAs from excess glucose from carbohydrates, and MUFAs from excess SFAs (Capra et al., 2006). However, animals (including humans) and plants differ in their ability to insert double bonds into fatty acid carbon chains to produce PUFAs. Humans can modify SFAs and MUFAs to synthesise PUFAs, but are the only mammals that cannot insert a double bond closer than seven carbons from the methyl end, and therefore cannot synthesise the longer chained *n*-3 and *n*-6 PUFAs (Russo, 2009). The conversion from one fatty acid to another requires three main types of enzymes – desaturase, which removes two hydrogen atoms from a carbon in the chain resulting in the insertion of a double bond, elongases, which adds two carbons to the chain, and beta oxidase, which removes two carbons from the chain. Desaturase are numbered with a delta number that refers to which carbon on the chain (this time counting from the carboxyl or acid end) the hydrogen atoms were removed from. Plants can insert double bonds at the 3rd, 6th and 9th carbon, e.g., at the 9th carbon in stearic acid (C18) with delta-9 desaturase to produce oleic acid (C18:1*n*-9), then a double bond at the 6th carbon in oleic acid with delta-12 desaturase to produce linoleic acid (LA; C18:2*n*-6), and then a double bond at the 3rd carbon with delta-15 desaturase to produce alpha-linolenic acid (ALA; C18:3*n*-3). Humans do not have delta-12 or 15 desaturase enzymes, which means they cannot convert the SFA stearic acid (C18) to LA or ALA, nor LA directly to ALA (Simopoulos, 2002).

The two most important or 'essential fatty acids' for human health are the long-chained LA and ALA. 'Essential' refers to the fact that the human body cannot produce these PUFAs and therefore they must be consumed in the diet, but also to how vital they are to

human health (Crupi, Marino, & Cuzzocrea, 2013). LA is found in seed oils such as safflower, sunflower and corn oil, whereas ALA is found in flaxseeds/linseeds, chia seeds, kiwifruit seeds, walnuts and some leafy green vegetables. LA is the precursor to three important *n*-6 PUFAs – dihomogamma linolenic acid (DGLA), arachidonic acid (ARA) and docosapentaenoic acid (DPA*n*-6). ALA is the precursor to three important *n*-3 PUFAs – docosapentaenoic acid (DPA*n*-3), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DGLA and EPA play important roles in producing eicosanoids (hormone-like signal lipids) which have anti-inflammatory and anti-thrombotic effects, whereas ARA produces eicosanoids, which have vaso-constriction, platelet aggregation, and pro-inflammatory effects (Brenna, Salem, Sinclair, & Cunnane, 2009). DPA*n*-3 and DHA play important roles in reducing inflammation and platelet aggregation, and DHA in vision and the function of the neuronal membrane (Dyall, 2015).

The conversion from LA and ALA to these six important PUFAs requires delta-5 and 6 desaturase as well as vitamins B3, B6, C and the minerals magnesium and zinc, which means that deficiencies in any of these nutrients can inhibit conversion. Humans have delta-5 and 6 desaturase, but the conversion rates vary from person to person due to genetics, sex and dietary factors. Stress levels and reduced digestive capacity, due to certain medical conditions or age, also play a role (e.g., Tully et al., 2003; Burdge, 2006; Denis et al., 2013). In terms of dietary factors, this conversion can depend on the availability of the starting fatty acid, on any competing fatty acids as well as whether there are high or low levels of the end-product fatty acid (Brenna et al., 2009). Competing fatty acids here refers to *n*-3 PUFAs and *n*-6 PUFAs competing for the use of desaturase enzymes, and therefore, if a higher amount of *n*-6 PUFAs are consumed in the diet than *n*-3 PUFAs, then the *n*-3 PUFAs will get converted at a lower rate (Brenna et al., 2009).

3.3 Long-chain omega-3 polyunsaturated fatty acids

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are very long chain *n*-3 PUFAs; EPA (C20:5*n*-3) has 20 carbon atoms in the carbon chain with five double bonds and DHA (C22:6*n*-3) has 22 carbon atoms with six double bonds. Both EPA and DHA are found in high amounts in oily fish such as salmon, mackerel and sardines, shellfish like mussels, fish eggs or roe, and certain types of marine algae (Capra et al., 2006). Fish can synthesise LA but not ALA, and obtain ALA from marine algae, plankton and krill which is then converted to EPA and DHA. EPA is also found in grass-fed beef and free-range eggs, and DHA is found in maternal milk. Both EPA and DHA can also be synthesised from ALA so neither are considered to be essential fatty acids, although due to the limited conversion rates of ALA to DHA, EPA and DHA are sometimes referred to as a 'conditionally essential fatty acids' (Arterburn, Hall, & Oken, 2006).

The process of converting the fatty acids from ALA to EPA through to DHA is shown in Figure 2. ALA (C18:3*n*-3) is first converted to stearidonic acid (C18:4*n*-3) by delta-6 desaturase (adds a double bond), then to eicosatetraenoic acid (C20:4*n*-3) by elongase (adds two carbons), then to EPA (C20:5*n*-3) by delta-5 desaturase. EPA is then converted to docosapentaenoic acid (DPA*n*-3) (C22:5*n*-3) and then to tetracosapentaenoic acid (C24:5*n*-3) using elongases, then to nisinic acid (C24:6*n*-3) using delta-6 desaturase, and finally to DHA (C22:6*n*-3) using beta oxidase. Only microalgae have shown the ability to convert DPA*n*-3 (C22:5*n*-3) directly to DHA (C22:6*n*-3) using delta-4 desaturase (Russo, 2009). The conversion from ALA to EPA to nisinic acid takes place in the liver within a cellular organelle called the endoplasmic reticulum, and the last steps to form DHA takes place within a peroxisome (Hulbert, 2003). Using isotope tracer studies, the conversion from ALA to EPA has been shown to be between 0.3% to 8% in men and up to 21% in women, whereas the conversion

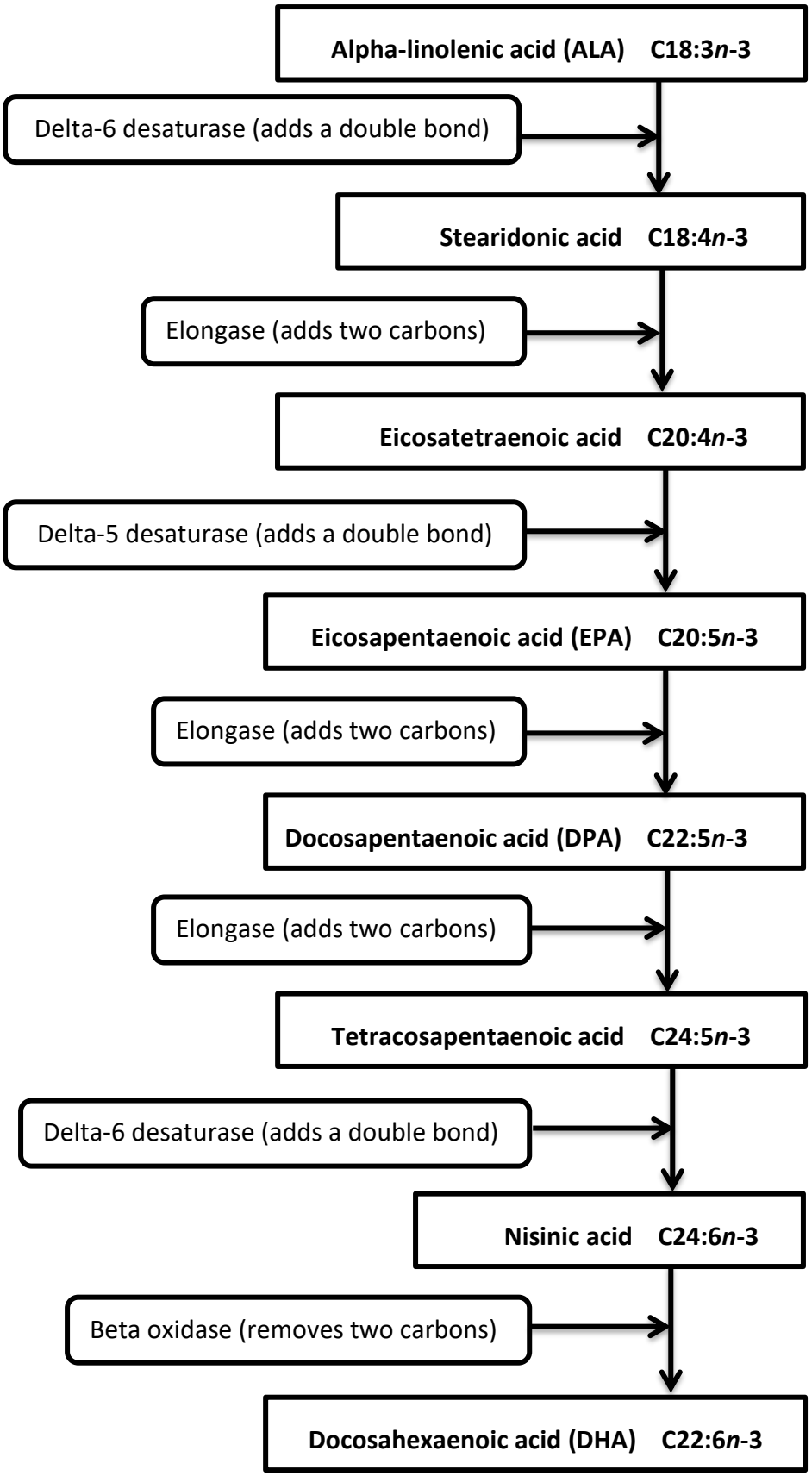

Figure 2. Conversion of alpha-linolenic acid (ALA) to docosahexaenoic acid (DHA)

from ALA to DHA is less than 4% in men (in some cases results in no change or a negative change), and up to 9% in women (Arterburn et al., 2006). The higher conversion rate in women is due to the influence of oestrogen (which increases desaturation), the lower amount used for energy production in women (via beta-oxidation), and the overall higher percentage of adipose tissue in women than in men – 15% vs. 23% in males (Burdge & Calder, 2005). The conversion from ALA to EPA and DHA is much less efficient in humans than in other animals, which is the main reason why the use of animals models is not useful is investigating the effects of these nutrients for human health-related outcomes.

Direct dietary or supplement intake of ALA has been shown to result in increases in EPA but not DHA, although several studies have shown increased DHA levels when LA levels are low (Burdge & Calder, 2005; Arterburn et al., 2006). In contrast, direct dietary or supplement intake of EPA and DHA results in correspondingly significant increases in EPA and DHA. Dietary EPA intake results in a linear response increase in EPA plasma phospholipid concentration, whereas dietary DHA intake results in a dose-dependent increase in DHA plasma phospholipid concentration up to 2 g per day, which reflects that DHA is the end product of the fatty acid metabolism. EPA supplementation does not always result in increased DHA levels; usually in an increase in EPA and DPA*n*-3 only, yet DHA intake does result in a linear response increase in EPA concentrations due to retro conversion at a rate of 0.4 g/100 g fatty acid per 1 g of DHA (Arterburn et al., 2006).

DHA exceeds EPA by 5 to 30-fold in most tissue types, and up to 700-fold in brain and retina tissue (Lin et al., 2012). EPA and DHA are found in the highest amounts in human tissue within the brain, retina, adrenal glands and sperm (Stillwell & Wassall, 2003). EPA is found in smaller quantities than DHA, but in more tissue types. EPA is found in cholesterol, triglycerides and phospholipids, whereas DHA is mostly found in phospholipids within cell

membranes (Simopoulos 1991; Kuratko, & Salem, 2009; Lin et al., 2012). In contrast, the amount of ALA in phospholipids is less than 0.5% of the total fatty acids, which suggests that ALA has very minimal effect on the function of cell membranes (Burdge & Calder, 2005). Between 25 to 60% of ALA is oxidised for energy production, whereas DHA is quickly incorporated into plasma and phospholipid cell membranes (Brenna, 2002; Stillwell & Wassall, 2003). Both EPA and DHA are highly susceptible to peroxidation due to the multiple double bonds, which has both advantages and disadvantages for human health. For example, by attracting oxygen EPA and DHA allows oxygen to be captured and incorporated into haemoglobin within the blood; however, it results in the production of free-radicals due to the oxidation reaction at the double bond (Cole & Frautschy, 2010). The fact that housing these two fatty acids results in oxidative stress, and that converting ALA to DHA is very energy and resource taxing process, is taken as an indication of how important these two PUFAs are to the functioning of the biological system (Olbrich, Rawicz, Needham, & Evans, 2000; Stillwell & Wassall, 2003).

3.4 Eicosapentaenoic acid (EPA)

One of the main roles of EPA is in producing eicosanoids, which are a group of hormone-like signalling molecules. Eicosanoids are made from fatty acids with 18 and 20 carbons, including both ARA and DGLA, taken from the phospholipid cell membrane. EPA produces several types of eicosanoids including resolvins and hydroxyl fatty acids, which have anti-inflammatory, anti-thrombotic and vaso-dilatory effects. In contrast, ARA produces types of eicosanoids called prostaglandins (series 2) and leukotrienes, which have inflammatory, vaso-constricting, and coagulation effects. The picture is not this simple though; ARA also produces lipoxins and hydroxyl fatty acids, both of which are important

anti-inflammatory agents, and EPA contributes to producing prostaglandins (series 3) and leukotrienes which have inflammatory and smooth muscle contracting effects (Calder, 2009). The difference between the two types of prostaglandins is that those produced from EPA are much slower to react than the prostaglandins created from ARA, which are very potent and fast acting (Bradbury, 2011). The opposing effects of these two different types of eicosanoids are crucial in helping the body to regulate and modify immune and inflammatory processes (Calder, 2009).

EPA has been shown to reduce inflammation in a dose dependent manner. For example, Rees et al. (2006) showed that doses of EPA from 1.35 g to 2.70 g to 4.05 g per day resulted in incremental decreases in the production of the inflammatory prostaglandin series 2, particularly in relation to high EPA to ARA ratios. The study also found that the production of prostaglandins series 2 was significantly negatively associated with EPA content in phospholipids ($r = -.23$, $p = .01$). Rees et al. also found that older men incorporated EPA into plasma more readily than younger men, irrespective of *n*-6 PUFA intake, which the authors suggest reflects changes due to age in the ways fatty acids are used and stored. ALA, ARA, EPA and DHA all compete for positioning within cell membranes, therefore EPA's role in regulating ARA levels (especially when dietary intake of *n*-6 PUFAs are high) by competing for the same desaturase is crucial (Arterburn et al., 2006; Huang, 2010).

EPA and DHA also decrease inflammation by decreasing several types of cytokines, in particular interleukin 1 *β* (IL-1*β*) and tumour necrosis factor α (TNF-α) (James, Gibson, & Cleland, 2000). Cytokines are signalling proteins that are released from immune cells and result in increased inflammation. IL-1*β* and TNF-α have been implicated in several chronic inflammatory conditions including rheumatoid arthritis (RA) and inflammatory bowel

disease (Calder, 2006). A meta-analysis by Goldberg and Katz (2007) of 17 *n*-3 PUFA RCTs in patients with RA found that *n*-3 PUFAs significantly reduced duration of morning stiffness, the number of painful/tender joints and consumption of nonsteroidal anti-inflammatory drugs (NSAID) after three months of supplementation. Another meta-analysis by Lee, Bae and Song (2012) did not find a significant reduction in pain-related symptoms in patients with RA, but found that 2.7 g of EPA + DHA per day for at least three months significantly reduced the use of NSAIDs. The authors note the significance of this finding is based on concerns around the adverse effects of NSAID such as increased cardiovascular risk and gastrointestinal damage (Lee, Bae, & Song, 2012).

3.5 Docosahexaenoic acid (DHA)

DHA has two more carbons and one more double bond than EPA, making it more unsaturated and therefore more dispersed and flexible in its structure and function (Feller, Gawrisch, & MacKerell, 2002; Valenzuela, 2009). DHA is involved in producing several very important intra and extracellular signalling molecules (Hamilton, Hillard, Spector, & Watkins, 2007). Where EPA produces eicosanoid signalling molecules such as prostaglandins, resolvins and leukotrienes, DHA produces docosanoids and docosatrienes such as maresins and a particular type of resolvin that generates neuroprotectins (Calon, 2011). Maresins and neuroprotectins have potent anti-inflammatory, neuro-protective and tissue regeneration effects (Valenzuela, 2009). DHA-derived neuroprotectin D1 has been shown to mediate apoptosis proteins and induce cell survival against oxidative stress in human retinal pigment cells, while maresin 1 has been shown to stimulate tissue regeneration and reduce pain (Colas, Shinohara, Dalli, Chiang, & Serhan, 2014; Calandria et al., 2015).

DHA makes up between 30 to 50% of the fatty acids in the synaptosomal membrane, mostly in the form of ethanolamine head groups within phospholipid membranes (Conquer, Tierney, Zecevic, Bettger, & Fisher, 2000; Tully et al., 2003). These ethanolamine head groups are a major constituent of synaptic vesicles, which store neurotransmitters, and play an important role in membrane fusion events within the synaptic cleft (Teague, Fuller, Rand, & Gawrisch, 2002). DHA influences the structure and function of the neuronal membrane in several different ways, namely by forcing cholesterol out of the membrane structure and by increasing membrane permeability to water and other substances (Olbrich et al., 2000; Stillwell & Wassall, 2003; Freemantle et al., 2006). Cholesterol levels increase within the cell membrane with age, and where cholesterol reduces membrane fluidity within the cell membrane structure, DHA increases fluidity (Yehuda et al., 2002). This ability to alter the movement of cholesterol in and out of the cell membrane may help to explain the important role of DHA in the brain with age. By increasing membrane fluidity and permeability, DHA also contributes to increasing the rate of protein to protein interaction within the cell membrane, and influences signal transduction and transmission from neuron to neuron (Valenzuela, 2009; Cunnane et al., 2009).

3.6 Evidence for the cognitive effects of EPA and DHA

The ability to assess EPA and DHA intake with the use of Food Frequency Questionnaires (FFQ) and to accurately measure levels of EPA and DHA within the body has been crucial to understanding the cognitive effects of *n*-3 PUFAs. The main issue with using FFQs as a measure of fatty acid levels is that they rely on the accuracy, memory and willingness of participants to report dietary intake, although a study involving 273 older adults with mild to moderate cognitive impairment found that interviewer administration

can increase the validity of FFQs (Sun, Ma, Campos, Hankinson, & Hu, 2007; Arsenault et al., 2009). The accuracy of food composition databases and age-related differences in how *n*-3 PUFAs are digested and metabolised also pose problems for using FFQs. Adipose tissue levels of DHA are considered the most accurate reflection of dietary and supplement intake; however accessing this type of tissue can be difficult, whereas accessing blood is much easier. Blood is composed mostly of serum (non-cell component of blood without the clotting factors), plasma (serum plus the clotting factors), white blood cells and erythrocytes or red blood cells (RBC), with serum, plasma and RBCs the most commonly used to measure levels of EPA and DHA (Tan et al., 2012). Both plasma and RBC levels of DHA correlate well with dietary intake and brain tissue levels, the difference being that plasma and serum levels reflect recent intake (weeks) and RBC levels reflect long term intake (months) (Katan et al., 1997; Kuratko & Salem, 2009). A large study with 306 women aged 43 to 69 found that both plasma and RBC levels were associated with EPA and DHA intake, with RBC fatty acid levels being better than plasma (Sun et al., 2007).

The omega-3 index has been used an *n*-3 PUFA biomarker for assessing the health risks associated with low levels of *n*-3 PUFAs, and as a way to quantify a low vs. high or deficient vs. sufficient level of *n*-3 PUFAs. The omega-3 index is calculated by taking the sum of EPA and DHA concentrations then dividing by total % fatty acid levels. The omega-3 index has been primarily used as a biomarker for coronary health disease risk (where an index score above eight is associated with the greatest cardio protection and an index score below four the least protection), but it is increasingly being used for assessing risk for dementia and cognitive decline (Harris & von Schacky, 2004). For example, Pottala et al. (2014) found that higher omega-3 index scores correlated with larger total brain and hippocampal volume in 1,111 postmenopausal women.

3.6.1 Brain and blood levels of EPA and DHA

The levels of fatty acids in brain tissue are made up of approximately 50% PUFAs, in a roughly 1:1 DHA to ARA ratio (Denis et al., 2013). The amount of DHA to ARA is higher in grey matter but lower in white matter, although the ratio of these two fatty acids appears to change with age (Bradbury, 2011). For example, a study comparing the brain tissue of 2 year olds through to healthy 80+ year olds found that both DHA and LA increase with age, whereas ARA and all other PUFAs decrease with age (Carver, Benford, Han, & Cantor, 2001). Despite the increase in LA, the study found that LA metabolites such as DGLA (C20:3*n*-6), ARA (C20:4*n*-6) and DPA*n*-6 (C22:5*n*-6) decreased with age, which the authors suggest may reflect a reduced conversion rate possibly due to a lack of desaturase enzymes. This study also investigated the relationship between cerebral cortex fatty acid levels and RBC fatty acid levels, and found a significant relationship in those over 18 years of age particularly for palmitic acid (C16) but also for DHA. In contrast to the increase in DHA with age in healthy brain tissue, several plasma, RBC and brain analysis studies of older adults with AD and MCI have shown a decrease in DHA levels.

Conquer et al. (2000) tested plasma fatty acid levels in 19 older adults with AD, 10 older adults with other forms of dementia (OD), 36 with CIND and 19 controls, and found decreased levels of DHA, total *n*-3 PUFAs and *n*-3:*n*-6 ratio in older adults with AD, OD and CIND. Tully et al. (2003) found that 139 older adults with AD (otherwise well-nourished and generally physically healthy) had approximately half the amount of serum DHA than the control group, as well as lower levels of EPA and *n*-3 PUFAs and higher levels of SFAs. Tully et al. also found higher ALA:DHA ratios in older adults with AD compared to controls indicating a lower conversion rate, perhaps due to a lack of enzymes or co-factors. Roberts et al. (2010) computed odds ratios for risk of MCI in 1,233 non-demented older adults and found a

significant dose-dependent decrease in risk for those with a high dietary intake of *n*-3 PUFAs (OR = 0.62) and total PUFAs (OR = 0.44) as well as a high ratio of combined PUFAs + MUFAs:SFAs (OR = 0.56) compared to those in the lowest tertiles for dietary intake. Lopez, Kritz-Silverstein, and Barrett-Connor (2011) found that higher levels of plasma and dietary DHA were significantly associated with reduced odds of AD and all other causes of dementia in 266 older adults including 72 with dementia. Milte et al. (2011) found lower EPA levels as well as higher DGLA, ARA and DPA *n*-6 levels in older adults with MCI compared to healthy controls. Tan et al. (2012) found that lower RBC levels of DHA and EPA in late middle age were associated with smaller brain volumes (equivalent to 2 years of brain ageing) and vascular patterns of cognitive impairment.

A meta-analysis by Lin et al. (2012) of 10 studies involving healthy controls, 481 older adults with dementia and 363 older adults with pre-dementia syndrome (MCI and CIND) found a significant decrease in EPA, DHA and total *n*-3 PUFAs in older adults with dementia, and a significant decrease in EPA but not DHA or total *n*-3 PUFAs in older adults with pre-dementia. The authors suggest the lower EPA levels but not DHA in MCI and CIND indicates a possible link between EPA and risk for early cognitive decline, and may even indicate the use of EPA levels as a biomarker for cognitive decline (Lin et al., 2012). Several studies have not found significantly lower DHA or *n*-3 PUFAs levels in older adults with AD, CIND, ARCD or MCI compared to healthy older adults (e.g., Cherubini et al., 2007; Castellano, Chouinard-Watkins, Brenna, Meyer, & Cunnane, 2011). One study even found higher EPA levels in older adults with CIND or MCI and higher levels of DHA, *n*-3 PUFA and total PUFA levels in those with dementia (Laurin, Verreault, Lindsay, Dewailly, & Holub, 2003). Whether low EPA and DHA levels are a risk factor or a marker for AD is currently unknown. Low levels of EPA and

DHA could be due to low dietary intake, reduced conversion or synthesis due to reduced desaturation or due to the disease process itself (Tully et al., 2003).

3.6.2 Epidemiological evidence

Numerous prospective studies have shown an association between fish consumption and a lower risk of or slower rate of cognitive decline. The Rotterdam study with 5,386 non-demented older adults found that fish consumption was inversely associated with an incidence of dementia, whereas SFAs and cholesterol levels were associated with an increased risk for dementia (Kalmijn et al., 1997). A 10-year follow-up analysis however, found that there was no longer an association between consumption of fish and *n*-3 PUFAs and long-term dementia risk (Devore et al., 2009). A prospective cohort with 1,674 older adults aged 68 and over without dementia found a significant trend between eating fish and seafood and a decreased incidence of dementia (Barberger-Gateau et al., 2002). A prospective study involving 815 older adults without AD found that those who ate fish at least once a week had 60% less risk of AD, and that both DHA and *n*-3 PUFA intake were associated with a reduced risk of AD (Morris et al., 2003). A large epidemiological study involving 2,950 non-demented older adults in France found that those who ate fish or seafood once a week had a significantly reduced risk of dementia as did moderate alcohol drinkers (Larrieu, Letenneur, Helmer, Dartigues, & Barberger-Gateau, 2004).

A prospective cohort study with 6,158 older adults over the age of 65 found that fish consumption, but not *n*-3 PUFA intake, was associated with slower cognitive decline with age (Morris et al., 2005). Another prospective follow-up study with 899 older adults as part of the Framingham Heart Study found that high plasma DHA levels (equivalent to three servings of fish per week) were associated with a significant reduction in all cause dementia

risk (Schaefer et al., 2006). A prospective cohort of older men found that fish consumers had less cognitive decline after the 5 and 10-year follow up, and a dose-dependent relationship between intake of *n*-3 PUFAs, EPA and DHA and cognitive decline (van Gelder, Tijhuis, Kalmijn, & Kromhout, 2007). A large US based prospective diet pattern analysis study involving 2,148 older adults found that a diet high in *n*-3 PUFAs, *n*-6 PUFAs, vitamin E, and folate, but with lower SFA levels, was significantly associated with reduced AD risk, whereas the nutrients individually were not (Gu, Nieves, Stern, Luchsinger, & Scarmeas, 2010). The study also found that older adults who were current smokers and less educated were less likely to adhere to a diet high in these nutrients, and that men were less likely to adhere than women.

Several cross-sectional studies have shown an association between fish consumption and better cognitive performance. A cross-sectional, population-based study with 1,613 older adults from 45 to 70 years found that fatty fish consumption was associated with a reduced risk of impaired cognitive function, whereas intake of cholesterol and saturated fatty acids was associated with an increased risk of impaired cognitive function (Kalmijn et al., 2004). A large cross-sectional study with 2,031 older adults between 70 and 74 years of age found that a diet high in fish and fish products was associated with better cognitive performance in a dose dependent manner, particularly for non-processed lean and fatty fish (Nurk et al., 2007). Baseline data from a large study with 867 non-demented older adults between 70 and 79 years of age also found a positive association between high levels of fish consumption, particularly for oily fish, and higher scores in cognitive tests (Dangour et al., 2009). In contrast, a study with 1,025 older men from the Veterans Affairs Normative Aging study did not find an association (the model was adjusted for age, educational level, BMI, smoking behaviour, alcohol intake, diabetes and SFA intake) between fatty fish consumption

nor *n*-3 PUFA intake with cognitive performance (van de Rest et al., 2009).

It appears the presence or absence of the APOE ε4 allele may be a modulating factor between fish consumption and cognitive decline. A longitudinal epidemiologic study with 915 older adults found that one meal of fish or seafood per week was associated with a slower decline in semantic memory, with slower rates of decline in global cognition, episodic memory and perceptual speed in APOE ε4 carriers, but not in non-ε4 carriers (van de Rest et al., 2016). van de Rest et al. also found that higher intake of ALA was associated with slower global cognitive decline in ε4 carriers. In contrast an observational follow-up study with 120 older adults found that DHA and total *n*-3 PUFA concentrations were associated with cognitive benefits, but only significantly in non-ε4 carriers when controlled for sex and IQ at age 11 (Whalley et al., 2008). Two other studies found that consumption of fatty fish twice a week (but not fried lean fish) was associated with a reduced risk for dementia for non-ε4 carriers but not ε4 carriers (Huang et al., 2005), and that daily consumption fish and *n*-3 PUFA oils was associated with a decreased risk for dementia among non-ε4 carriers (Barberger-Gateau et al., 2007). A possible explanation for these findings comes from a study by Plourde et al. (2009) who found that while ε4 carriers had significantly higher baseline EPA and DHA levels in plasma, the incorporation of EPA and DHA from a fish oil supplement was decreased in ε4 carriers, and in fact EPA plasma levels did not increase in ε4 carriers despite a very high dose of 1900 mg of EPA per day for six weeks.

The discrepancies between the evidence for fish consumption, for *n*-3 PUFA, EPA and DHA intake and *n*-3 PUFA levels suggests that the protective effect of fish consumption may be from more than the *n*-3 PUFA content. For instance, fish may provide a preventative effect via the vitamin E and D content of oily fish, as a form of easily digested protein or as part of an overall healthier diet and lifestyle. Issues related to the assessment and diagnosis

of cognitive decline as well as genetic, demographic, cultural and lifestyle factors are also likely to play a part (Dacks, Shineman, & Fillit, 2013). For example, Barberger-Gateau et al. (2002) found that fish consumption was higher in participants with higher education, and that the protective effect of weekly fish and seafood consumption was partly explained by higher education.

3.7 Mechanisms for preventing cognitive decline

There are several proposed explanations for how DHA, EPA and *n*-3 PUFAs work to decrease or prevent cognitive decline pathology. Firstly, *n*-3 PUFAs may work by decreasing amyloid *β* deposits and the effects of these deposits on oxidative stress, inflammation and apoptotic cell death. Secondly, they could work by providing protective vascular and cardiovascular effects. Thirdly, *n*-3 PUFAs may benefit by directly reducing inflammation by inhibiting pro-inflammatory mediators. Fourthly, by the specific role of DHA within the neuronal cell membrane (Lin et al., 2012), and lastly via the APOE gene.

To date there have been no human studies specifically looking at the effects of *n*-3 PUFAs on amyloid *β* deposits. The majority of evidence used to support the mechanisms by which *n*-3 PUFAs prevent cognitive decline comes from animal studies. However, there is some human-based research, including studies that show that *n*-3 PUFAs can help to reduce the effects of oxidative stress and protect human neural cells. The brain is particularly prone to oxidative stress due to a high content of multiple-bond PUFAs, in particular DHA and ARA, and because of the high production of free radicals due to the brain's requirement for high amounts of oxygen and high consumption of glucose and low anti-oxidant protective defences (Floyd & Hensley, 2002). A RCT involving 59 type 2 diabetic adults between 40 and 75 years of age found that urinary F2-isoprostanes, a measure of lipid peroxidation, was

significantly decreased by a 6-week course of EPA or DHA supplement (Mori et al., 2003). Another trial involving 162 adults found that 3-months of an EPA + DHA supplement significantly decreased plasma 8-isoPGF2a, which is a biomarker of lipid peroxidation and oxidative stress (Nälsén et al., 2006). Another study by Lukiw et al. (2005) found that DHA-derived lipid mediator neuroprotectin reduced amyloid *β* secretion in cytokine-stressed human neural cells.

The protective vascular and cardiovascular effects of *n*-3 PUFAs are thought to be primarily via the anti-thrombotic and vaso-dilatory effects of eicosanoids and docosanoids (Lin et al., 2012). EPA and DHA have been shown to reduce blood platelet reactivity, reduce plasma viscosity, reduce triglycerides and VLDL levels by increasing HDL cholesterol and to have an inhibitory effect on atherosclerosis and inflammation via inhibition of smooth muscle cell proliferation and altered eicosanoid synthesis (Holub & Holub, 2004). A RCT in adults with hypercholesterolemia (high cholesterol) found that an EPA + DHA supplement significantly improved brachial artery flow-mediated dilation and significantly reduced triglycerides (Goodfellow, Bellamy, Ramsey, Jones, & Lewis, 2000). In a RCT involving adults awaiting carotid endarterectomy surgery (to remove arterial plaque build-up) showed that those given the EPA + DHA supplement compared to the sunflower oil and control groups had plaques with well-formed, thick fibrous caps (less likely to rupture) indicating an enhanced stability of the atherosclerotic plaques (Thies et al., 2003). Thies et al. also found those given the EPA + DHA supplement had a reduced number of macrophages in the plaques (indicating less infection) and no signs of inflammation. By way of inhibiting platelet aggregation, EPA and DHA are also thought to increase blood flow and supply of nutrients to the brain as well as increase the removal of toxic metabolites and proteins from the brain (Jicha & Markesbery, 2010).

EPA, DHA and *n*-3 PUFAs have been shown to directly reduce inflammation by inhibiting pro-inflammatory mediators such as inflammatory cytokines including interleukins, tumour necrosis factor and mitogens and by inhibiting the production of ARA-derived pro-inflammatory eicosanoids (Holub & Holub, 2004). For example, the OmegAD trial involving 174 older adults with AD found that higher plasma levels of EPA and DHA were associated with a decrease in the pro-inflammatory factors interleukin-1b, interleukin-6 and granulocyte-stimulating factor by immune cells (Vedin et al., 2008). Another study involving dialysis patients found that a supplement containing DHA + gamma tocopherol (vitamin E) significantly decreased plasma interleukin-6 concentrations and total white blood cell count including neutrophils – all of which are signs of infection and inflammation (Himmelfarb et al., 2007).

DHA plays a significant role within the neuronal cell membrane where it affects nerve conduction, neurotransmitter release and neurotransmitter reuptake (Morris et al., 2003). DHA is also an important regulator of brain glucose uptake, possibly by selectively affecting the activity of glucose transporters (Freemantle et al., 2006). EPA and DHA are also involved in the regulation of gene expression of cells via peroxisome proliferator activated receptors and sterol regulatory element binding protein, allowing the cell to metabolize fuel according to the availability in the external environment (Pégorier, May, & Girard, 2004). There is evidence to support a homeostatic mechanism that controls how DHA is used, stored and conserved within the body, especially when dietary intake is low. For example, Rosell et al. (2005) compared plasma proportions of dietary fatty acids, including LA, ALA, EPA and DHA in meat-eating, vegetarian and vegan men, and found lower EPA and DHA levels in vegetarians than meat-eaters, and even lower levels in vegans. The authors noted that the difference between vegetarians and vegans might be the result of the consumption of eggs,

a source of EPA and DHA. Rosell et al. found that the vegan group showed stable DHA concentrations, regardless of whether it had been one or 20 years since becoming a vegan, indicating that humans can convert ALA to DHA when there is a deficiency or a diet low in *n*-6 PUFAs, as the ratio of LA:ALA was inversely correlated with EPA and DHA.

As of yet, it is not clear how *n*-3 PUFAs interact with the APOE ε4 allele in cognitive decline. The first possibility is via cholesterol homeostasis as APOE is one of the main carriers of cholesterol within the brain. Research shows that ε4 carriers have the lowest levels of APOE in the blood, brain and CSF as well as the highest levels of plasma and LDL cholesterol, most probably because the ε4 allele is the least effective in lipid membrane turnover and the most inefficient at delivering PUFAs, particularly DHA, to glia, neurons and membranes (Lane & Farlow, 2005; Barberger-Gateau et al., 2011; Poirier et al., 2014). Minihane et al. (2000) investigated the effects of a fish oil supplement (equivalent to 3000 mg of EPA and DHA per day) on cholesterol levels in ε2, ε3 and ε4 carriers and found that both ε3 and ε4 carriers showed a decrease in fasting circulating triglycerides, but not as much as ε2 carriers who also showed a substantial decrease in lipoprotein lipase (a lipoprotein that breaks down fats) and postprandial (after food) circulating triglycerides.

The other possibility is via neurodegenerative processes, where ε4 has been associated with faster myelin breakdown, greater hippocampal atrophy and reduced neuroplasticity capacity along with greater inflammation and oxidative stress (Blennow et al., 2006; Fjell & Walhovd, 2010; Barberger-Gateau et al., 2011). APOE is involved in the binding, clearance and metabolism of the amyloid *β* peptide and in tau protein metabolism, where ε4 appears to have the lowest affinity and therefore be the least effective of the three alleles thereby "acting as a pathological chaperone" (Lane & Farlow, 2005, pg. 962). The fact that ε4 carriers seem more vulnerable to neuronal damage and respond differently to *n*-3 PUFA intake

suggests that at best *n*-3 PUFAs may only provide a neuro-protective effect to prevent further damage in ε4 carriers, but not necessarily a nootropic effect with a noticeable effect on cognition.

3.8 Summary

The pharmacological and epidemiological evidence for the role of *n*-3 PUFAs in cognition in the mid to late stages of life is relatively strong, however some inconsistencies mean that no conclusive links can be drawn. Despite these inconsistencies, it is clear that humans require LA, ALA, ARA, EPA and DHA for optimal health, and that there is a need to understand which foods best provide these nutrients in a way that maximises the brain's ability to perform and protect itself. It is also clear that the issue with fatty acids is not so much the quantity that is consumed, but more the ratio of different types of fatty acids consumed, with the Western-style diet having too much saturated fatty acids and *n*-6 PUFAs compared to *n*-3 PUFAs. There has been an increase in interest in the use of *n*-3 PUFA supplementation for the treatment of cognitive and behavioural conditions; however, it is still unclear at what stages it is most efficacious. Measuring the cognitive effects of *n*-3 PUFAs may be difficult in older adults particularly those with a high cognitive reserve and those who are carriers of the APOE ε4 allele, and because of age-related changes in digestion and metabolism, and the homeostatic mechanisms by which DHA is used and conserved within the brain.

CHAPTER 4

FISH OIL SUPPLEMENT USE IN NEW ZEALAND

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Note. This manuscript does not include the results from Section 2, Question 12 (see Appendix C) which asked whether respondents would be willing to have a blood sample taken as part of a research study.

Abstract

Objective: The aims of the survey were to determine: (i) the percentage of fish oil supplement users in a sample population; (ii) why people take fish oil supplements; (iii) where fish oil supplements are stored as well as the average daily dosage; (iv) what dietary and lifestyle behaviours are associated with fish oil supplement use

Design: An online cross-sectional survey.

Setting: New Zealand.

Respondents: A total of 334 New Zealand residents over the age of 18.

Results: Fish oil supplements were taken by 21.9% of respondents. Reasons for taking fish oil supplements were - 72.6% for 'general well-being', 54.8% to 'improve brain function', 31.5% for 'pain/inflammation', 12.3% to 'lower cholesterol levels' and 11% for 'a dietary insufficiency'. Approximately 26% of fish oil users reported taking a dose of fish oil supplements that would meet the recommended daily intake of 400-600 mg combined docosahexaenoic acid and eicosapentaenoic acid, and only 6.8% of fish oil users reported storing their fish oil supplements in the refrigerator. After controlling for other characteristics including age, gender, ethnicity and body mass index, fish oil supplementation use was most likely among respondents who already eat oily fish and least likely in respondents who regularly eat nuts and seeds.

Conclusions: Fish oil supplements are a commonly used supplement in New Zealand, yet questions remain about the role of these supplements in improving health outcomes. Safety issues related to manufacturing and storage conditions indicate that there is an urgency in answering these questions.

Introduction

Supplement use surveys indicate that there has been an increase in the use of fish oil supplementation over the last 10-20 years (Kantor et al., 2016; Fish oil, multivitamins still most used supplements, 2015). This popularity may be due to a number of studies showing the beneficial effects of omega-3, polyunsaturated fatty acids (*n*-3 PUFA) on learning difficulties and developmental conditions in school age children (Richardson et al., 2012; Portillo-Reyes et al., 2014; Bloch & Qawasmi, 2011), on mood and cognition (Sublette, Ellis, Geant, & Mann, 2011; Grosso et al., 2014; Stonehouse et al., 2013; Yurko-Mauro et al., 2010), as well as cardiovascular and arthritic conditions (Delgado-Lista et al., 2012; Miller, Van Elswyk, & Alexander, 2014; Lee, Bae, & Song, 2012) in young, middle-aged and older adults. Despite these positive results, the majority of *n*-3 PUFA clinical trials have not found a significant improvement in outcomes, and several design issues have been proposed such as an insufficient dose, an insufficient duration, unknown baseline *n*-3 PUFA status, and the lack of a uniform biomarker to explain these negative and at times contradictory results (Stonehouse, 2014).

Fish oil supplements contain two types of *n*-3 PUFAs – docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). DHA and EPA are found in oily fish such as salmon, tuna, anchovies and sardines as well as krill and some shellfish. DHA and EPA and the plant derived alpha-linolenic acid (ALA) which is found in seeds and nuts, are important nutrients for bodily functioning, particularly brain functioning, due to their anti-inflammatory, anti-thrombotic and vaso-dilatory properties (Parletta, Milte, & Meyer, 2013). DHA is one of the most dominant structural lipids in the brain, where it plays a protective role, and improves the fluidity of the neuronal cell membrane thereby influencing neuronal cell signalling (Bradbury, 2011; Dyall, 2015). While the evidence for *n*-3 PUFAs, DHA and EPA as safe

therapeutic agents is steadily growing, it is still unclear what role they play in maintaining brain function from childhood to older age (Karr, Alexander, & Winningham, 2011).

In New Zealand (NZ), the Ministry of Health recommends an 'adequate intake' (AI) of 800 mg of ALA and 90 mg of combined DHA+EPA for women over 19 years of age and an AI of 1300 mg of ALA and 160 mg of combined DHA+EPA for men over 19 years of age, whereas the upper level at the 90th percentile, which provides a 'potential benefit while still at a safe level', is set at 430 mg/day combined DHA+EPA for women and 610 mg/day for men (Capra, 2006). This upper level is consistent with recommendations by the National Heart foundation to eat fish twice a week which would provide between 400 and 600 mg of combined DHA+EPA per day (Heart foundation, 2016). Most fish oil supplements in NZ contain between 1000-1500 mg of fish oil per capsule, equivalent to between 300-700 mg of *n*-3 PUFAs with the amount of DHA and EPA varying between 100-360 mg of DHA and 100-540 mg of EPA.

There has been considerable media attention in NZ regarding fish oil supplements after a study showed that of the 32 fish oil supplements tested from retail and online stores within NZ, 69% of the products contained less than 67% of the EPA and DHA content specified on the label, and that oxidation levels were higher than recommended international standards (Albert et al., 2015). Both DHA and EPA consist of long carbon chains with multiple double bonds which makes them very sensitive to light and heat, and therefore depending on manufacturing and storage conditions they can easily become oxidised and rancid. The New Zealand Institute for Plant and Food Research state that temperature is the number one contributing factor to the oxidation of oils (Miller, n.d.) and even though it is difficult for consumers to control or even access information about conditions during processing and transportation of supplements, it is generally

recommended that *n*-3 PUFA products including fish oils and ALA containing oils such as flaxseed and hemp seed oils are stored in opaque containers in a cool dark place such as a refrigerator.

The 2008/2009 New Zealand Adult Nutrition Survey contains the most recent information about fish oil supplementation use in NZ (New Zealand Adult Nutrition Survey, 2011). This survey showed that 30.7 % of the respondents (25.5% of males, 35.5% of females) were 'regular' users of supplements ('regular' meaning consumed a supplement at least once a week). The most frequently consumed supplements were 'oils' (fish oils, omega 3, 6 and/or 9, flax/linseed oils and evening primrose) at 16.4% (13.7% of males, 18.9% of females), followed by multi-vitamins/minerals at 14.8%. In terms of 'oils' users, age was a significant factor for female 'oils' users, where 6.9 % of females aged 15-18 took oils, compared to 14.3% of females aged 19-30, 17.9% of females aged 31-50 and 24.8% of females aged 51 plus. The 2011-12 Australian Health Survey showed that 29% of respondents (33% of females, 24% of males) reported taking at least one dietary supplement on the previous day (Australian Health Survey, 2012). Multi-vitamin/multi-mineral supplements were the most common dietary supplement at 16%, followed by fish oil supplements by 12% of the population. The highest percentage of fish oil users were in the 51+ age groups.

The associations between supplementation use and demographics or other health behaviours have been extensively studied (Lyle, Mares-Perlman, Klein, Klein, & Greger, 1998; Kirk, Cade, Barrett, & Conner, 1999; Satia-Abouta et al., 2003; Archer et al., 2005; Rock, 2007; Nahin et al., 2007). These studies have consistently found that supplement use is more common among women, older and more educated respondents, and in respondents who have a low to normal body mass index (BMI), a moderate to high level of physical

activity, who drink low to moderate amounts of alcohol and who are less likely to be a current smoker of cigarettes. When studies have included dietary measures they have shown that supplement users generally report higher levels of dietary nutrient intakes and in some cases exceed the recommended daily allowances. The 'inverse supplement hypothesis' was proposed to explain why people who are most likely to take supplements were often the people that need them the least. In support of this hypothesis a study found that 43% of a sample of Australians over the age of 51 consumed fish oil supplements, and that these fish oil consumers had a higher daily intake of finfish or seafood than those who were non-supplement consumers (Grieger, Miller, & Cobiac, 2013). Reasons for why people take supplements vary, generally either to 'improve overall health' or for 'general well-being' as a preventive measure or to treat a medical condition or a symptom such as cancer or pain. Respondents from the National Health and Nutrition Examination Survey indicated that they were most likely to use fish oil supplements for 'heart health or to lower cholesterol' (Bailey et al., 2013).

The current survey was designed to provide an update on the use of fish oil supplementation in a sample population of New Zealanders, and to determine why people use fish oil supplements. To date very few surveys have specifically asked fish oil users what dose they take and where the fish oils are stored. These questions were addressed as well as testing the hypothesis that fish oil users are likely to be more health conscious and therefore display healthier dietary and lifestyle habits.

Method

Survey population

This online survey was a cross-sectional analysis of a sample of the general population in New Zealand over the age of 18. The survey was advertised for a 6-month period from June until November 2015 on the Massey University School of Psychology mailing lists, the Massey University Facebook page, as well as through posters placed in public libraries, community centres and hospitals in main cities within New Zealand. All advertisements explained the purpose of the study and specified that anyone over 18 years of age could complete the survey. Respondents were instructed to click on a link or enter a URL to access the online survey questionnaire. The survey took an average of 9 minutes to complete.

Questionnaire design

The survey consisted of five sections; section 1 contained an information page with a consent question, section 2 contained demographic and general health questions, section 3 questions about fish oil supplement use, section 4 about general supplementation use and section 5 contained questions about dietary habits and exercise. The demographic questions in section 2 included age, gender, city/town of residence, ethnicity, highest qualification achieved and annual salary. The general health questions included self-reported health status, whether a current tobacco smoker or not, and current height and current weight, which were used to calculate body mass index (BMI). Section 3 began with the question “Do you take fish oil supplements?”. If respondents answered ‘yes’ they were classified as a ‘fish oil user’ and completed section 3 which included questions regarding frequency of fish oil supplement use, reasons for taking fish oil supplements as well as what dose of fish oils

respondents used and where respondents stored their fish oil supplements. Once completed, 'fish oil users' were directed to section 4 which began with the question "Do you take any other types of supplements?". If respondents had answered 'no' to taking fish oil supplements, they were classified as a 'non-fish oil user' and were automatically directed to section 4.

Section 4 of the survey asked respondents to specify what type of other supplements they took along with questions regarding frequency, reasons for supplement use, where the supplements were purchased, how much is spent per month on supplements, what influenced the decision to select a supplement and whether respondents told their GPs that they took supplements. Respondents were able to select 'I don't take any supplements' at the beginning of section 4 and were then directed to Section 5 which asked how often respondents ate oily fish, white fish, nuts and seeds, free range eggs/chicken/grass fed meat, as well as how many portions of fruit and vegetables respondents ate per day and how often respondents engaged in exercise for longer than 30 minutes at a time.

Statistical analysis

Statistical analysis of the data was conducted using SPSS version 24. Chi-square tests including Fisher's exact test were performed to assess demographic differences between supplement users and non-supplement users, and between fish oil users and non-fish oil users. Logistic regression was used adjusted for age, gender, ethnicity, BMI, qualifications, smoking status, salary, self-reported health status, dietary intake of oily fish and nuts/seeds to assess what factors predict fish oil supplement use. P values equal to or less than 0.05 were considered statistically significant.

Results

A total of 346 New Zealand residents completed the survey, 12 (3.5%) respondents were omitted because they did not complete section 3, resulting in 334 being included in the analysis. More than a half of respondents, 223 (66.8%) reported taking at least one type of supplement and 73 (21.9%) of respondents reported taking a fish oil supplement.

General demographics and supplement use

Table 3 shows the demographics of the respondents, as well as the supplement and fish oil users by demographic factors. Using a chi-square test for independence, there were no significant differences between supplement users and non-supplement users in any of the demographic characteristics. The only significant difference between fish oil users and non-fish oil users was for the salary category $150,000 plus per year, $\chi^2(7, p = 0.02)$. The sample was skewed in the demographic variables of age, gender and highest qualification gained – 194 (58.1%) of respondents were between 18-39 years of age, 262 (78.4%) of respondents were ‘female’ and 133 (39.8%) of respondents held a ‘post-graduate qualification’. This is probably due to the survey being posted on a psychology graduate email list The most common supplement used was a ‘multi-vitamin/mineral’ supplement (28.1 %), followed by ‘fish oil’ (21.9%), ‘Single mineral’- Magnesium (16.5%) and ‘Probiotics’ and ‘Single vitamin’- Vitamin C (15%).

In terms of cost, 31.7% of respondents spent between ‘0-$19’ (NZD) on supplements per month, although over 38.6% of respondents did not respond to the question. Supplements were purchased from a supermarket (29.9%), at a pharmacy (28.1%), at a health shop (17.7%) and online (12.9%). Factors influencing respondents’ decisions to purchase a supplement were 'quality of the product'(35.6%), ‘on sale/special price (26%)

Table 3. Demographic characteristics of supplement users and fish oil users

		Total sample		Supplement users		Fish oil users		
Background factors		*n*	%	*n*	%	*n*	%	χ^2
Gender	Male	59	17.7	37	16.6	12	16.4	
	Female	262	78.4	177	79.4	57	78.1	
	Other	13	3.9	9	4	4	5.5	
Age	18-29 years	111	33.2	67	30	22	30.1	
	30-39 years	83	24.9	59	26.5	17	23.3	
	40-49 years	49	14.7	35	15.7	11	15.1	
	50-64 years	74	22.2	51	22.9	18	24.7	
	65-85 years	17	5.1	11	4.9	5	6.8	
Ethnicity	NZ Euro/Pak	252	75.4	174	78	56	76.7	
	NZ Māori	23	6.9	12	5.4	3	4.1	
	Pacific Islander	4	1.2	1	0.4	0	0	
	Chinese	13	3.9	7	3.1	2	2.7	
	Indian	4	1.2	2	0.9	0	0	
	Other	38	11.4	27	12.1	12	16.4	
Education	HS attendance	11	3.3	8	3.6	4	5.5	
	HS completed	19	5.7	14	6.3	3	4.1	
	University Entry	26	7.8	16	7.2	4	5.5	
	Trade/Polytechnic	22	6.6	17	7.6	7	9.6	
	Diploma/Cert	27	8.1	21	9.4	7	9.6	
	Bachelor's degree	95	28.4	60	26.9	17	23.3	
	Postgraduate	133	39.8	86	38.6	31	42.5	
Salary	0-$14,999	83	24.9	56	25.1	21	30	
(per annum)	$15,000-$24,999	26	7.8	20	9	9	12.8	
	$25,000-$ 44,999	60	18	40	17.9	12	16.4	
	$45,000-$64,999	61	18.3	34	15.2	9	12.8	
	$65,000-$84,999	57	17.1	38	17	9	12.8	
	$85,000-$104,999	17	5.1	14	6.3	6	8.5	
	$105,000-$149,999	12	3.6	7	3.1	0	0	
	$150,000 plus	7	2.1	5	2.2	4	5.7	$P < .05$
BMI	Underweight	5	1.5	4	1.8	2	2.7	
	Healthy	183	54.8	118	52.9	41	56.2	
	Overweight	81	24.3	52	23.3	15	20.5	
	Obese	59	17.7	45	20.2	12	16.4	
Exercise	Never/Rarely	44	13.2	31	14	12	17.3	
	Monthly/fortnightly	17	5.1	7	3.1	2	2.9	
	1-2 times per week	97	29	67	30	16	23.1	
	3-4 times per week	95	28.4	59	26.5	23	33.3	
	5-6 times per week	45	13.5	35	15.6	9	13	
	Every day	30	9	19	8.5	7	10.1	
Smoker	Yes	31	9.3	17	7.6	4	5.5	
	No	303	90.7	206	92.4	69	94.5	

Note. Euro/Pak = European/Pakeha, HS = High school

Table 4. Characteristics of fish oil supplement users

		n	%
How often	Every day	39	53.4
	4-6 times per week	11	15.1
	1-3 times per week	7	9.6
	Several times per month	11	15.1
	About once a month	1	1.4
	Less than once a month	4	5.5
Length of time	Less than 3 months	14	19.2
	Between 3 to 6 months	12	16.4
	Between 6 to 12 months	8	11
	More than 12 months	39	53.4
Reason	General well-being	53	72.6
	To improve brain functioning	40	54.8
	For joint pain/inflammation	23	31.5
	Recommended by a health professional	13	17.8
	Was told about by friends/family	11	15.1
	To lower cholesterol levels	9	12.3
	Dietary insufficiency	8	11
	Read about them on internet	8	11
	Other	6	8.2
	Heard about them on TV/radio	4	5.5
	For skin health	3	4.1
	Read about them in a magazine	3	4.1
Daily dosage of fish oil	0 – 499 mg	1	1.4
	500 – 999 mg	2	2.7
	1000 – 1499 mg	18	24.7
	1500 – 1999 mg	23	31.5
	> 2000 mg	19	26
	No idea	2	2.7
	No response	8	11
Storage	In the pantry/food cupboard	35	47.9
	On my bench top	23	31.5
	In the refrigerator	5	6.8
	No response	5	6.8
	Bedroom	4	5.5
	Bathroom	1	1.4

and brand (21.9%). Forty-one percent said they take supplements for 'general well-being', 16.8% because they were prescribed by a health professional and 15.9% for a specific 'medical condition'. Ninety-five percent of supplement users take supplements at least once a month and 48.1% every day. Most respondents (62.8%) told their GP that they take supplements and 43.5% reported currently taking medications prescribed by a GP.

Fish oil supplement users

Table 4 shows that of the 21.9% of respondents who took fish oil supplements, 53.4% reported taking them every day and 53.4% reported having taken them for longer than 12 months. Reasons for taking fish oils were – 72.6% for 'general well-being', 54.8% to 'improve brain function', 31.5% for 'pain/inflammation', 12.3% to 'lower cholesterol levels' and 11% for a 'dietary insufficiency'. Around 26% of fish oil users are taking a dose of fish oil that would meet the daily recommendations for DHA and EPA, and 6.8% of fish oil users store their fish oil supplements in the refrigerator. After controlling for other characteristics (age, gender, ethnicity, BMI, self-reported health status, highest qualification, annual salary, smoking status, exercise, consumption of oily fish, and consumption of nuts and seeds) fish oil supplement use was most likely among respondents who eat oily fish (p<0.01), in respondents who identify as 'Other' in terms of gender (p<0.01) and who report an annual salary of '$150,000 plus' ($p$<0.01). Table 5 also shows that when controlling for all of these characteristics, fish oil supplement use was least likely in respondents with a highest qualification of 'university entrance' and 'diploma/certificate' (p<0.05), in respondents who eat nuts (p< 0.01), and in respondents with an annual salary of '$45,000-$64,999' and '$65,000-$84,999' (both p<0.01).

Table 5. Logistic regression model describing the relative probabilities of taking fish oil

Variable		*P* value	OR (95 % CI)
Gender	Male	<.001	1.00
	Female		1.66 (0.58, 4.79)
	Other		23.13 (2.74, 195.21)
Eats oily fish	Never	<.001	1.00
	Rarely		10.10 (1.33, 76.82)
	Once a month		19.45 (2.49, 152.12)
	Once a fortnight		9.06 (1.28, 64.16)
	1-2 times per week		15.16 (2.17, 105.98)
	> 2 times per week		5.67 (0.56, 57.26)
Qualification	HS attendance	<.05	1.00
	HS completed		0.09 (0.007, 1.09)
	University Entrance		0.08 (0.007, 0.85)
	Trade/Polytechnic		1.36 (0.13, 13.96)
	Diploma/Certificate		0.08 (0.007, 0.99)
	Bachelor's degree		0.20 (0.03, 1.51)
	Postgraduate degree		0.27 (0.37, 1.87)
Salary	0-$14,999	<.001	1.00
(per annum)	$15,000-$24,999		0.87 (0.24, 3.07)
	$25,000-$ 44,999		0.47 (0.17, 1.29)
	$45,000-$64,999		0.15 (0.04, 0.53)
	$65,000-$84,999		0.17 (0.05, 0.61)
	$85,000-$104,999		0.25 (0.04, 1.43)
	$105,000-$149,999		0
	$150,000 plus		46.88 (3.83, 573.62)
Eats nuts/seeds	Never	<.05	1.00
	Rarely		0.41 (0.02, 7.4)
	Once a month		0.04 (0.002, 0.86)
	Once a fortnight		0.02 (0.001, 0.41)
	1-2 times per week		0.15 (0.01, 2.1)
	> 2 times per week		0.33 (0.02, 4.42)

Note. HS = High school

Discussion

In this sample of 334 New Zealanders, 66.8% reported using supplements. This figure is more than double the number of supplement users in both the 2008/2009 New Zealand Adult Nutrition Survey and the 2011-12 Australian Health Survey. This figure is also higher than three NZ based supplement surveys, which showed 16.6%, 38.1% and 47% of respondents reported using supplements (Allen et al., 2000; Nicholson, 2006; Bacon, 2011).

Almost 30% of respondents in the current survey purchased their supplements from a supermarket, compared to only 2.5% of respondents from a 2006 NZ survey (Bacon, 2011). This may represent a change in shopping habits, where the convenience and lower prices of a supermarket may be deciding factors.

Fish oil supplements were used by 21.9% of respondents in the current survey, second only to multi-vitamin/mineral supplements at 28.1%. This finding is consistent with the 2011-12 Australian Health Survey, although in lower numbers in this survey at 12% and 16% respectively. In the 2008/2009 New Zealand Adult Nutrition Survey, 'oils' were the most commonly used supplement at 16.4%, where 'oils' included fish oil supplements as well as evening primrose and flaxseed oils. Again this figure is lower than in the current survey with an equivalent total of approximately 25% (*n* = 83) of respondents using fish, evening primrose or flaxseed oils. The higher percentage of fish oil users in the current survey compared to the 2008/2009 New Zealand Adult Nutrition survey indicates that fish supplement use is increasing in NZ. Fish oil users in the current study reported taking fish oil supplements for 'general well-being'(72.6%), to 'improve brain functioning' (54.8%), for 'pain/inflammation'(31.5%), to 'lower cholesterol levels'(12.3%) and for 'dietary insufficiency'(11%), indicating that fish oils are used to both prevent and treat health conditions.

The 2008/2009 New Zealand Adult Nutrition Survey showed that 24.8% of females aged 51 plus took 'oils' compared to 31.5% of fish oil users over the age of 50 in the current study, indicating an increase in use in older adults. A large US survey showed that 65+year olds were more likely to be fish oil users than 20-39 and 40-64 age groups, and that the 65+ age group showed the biggest increase in fish oil supplement use from the 1999-2000 to the 2011-2012 survey (Kantor et al., 2016). A large population-based study from the United

Kingdom (UK) showed that 'oils', in particular fish liver oil, are the most commonly used supplement in older adults in the UK, and that 'oils' are more commonly used by older men with the highest BMI and in lower social classes, and in older women with the least healthy diets (Denison et al., 2012). The authors suggest that this high prevalence maybe due to the widespread use of fish liver oil in childhood, indicating a habitual pattern of use, possibly related to dietary insufficiency and socio-economic status. The current survey showed that 43% of fish oil users over 50 years of age take fish oils to 'improve brain functioning' and 65% for 'general well-being', whereas only 17% take fish oils to 'lower cholesterol levels'. The fact that 60% of fish oil users under the age of 50 years of age also reported using fish oil to 'improve brain functioning' indicates that both young and older adults believe fish oil supplementation can provide some benefit here.

The increase in fish oil supplement use over the last two decades may be due to the link drawn between fish consumption and fish oil supplementation use, and improvements in cognition and memory. In older adults, epidemiological research has shown positive associations between fish consumption and both higher scores in cognitive tests (Dangour et al., 2009) and a slower rate in cognitive decline (Morris et al., 2005) in older adults, and yet clinical trials have shown mixed results in healthy older adults (van de Rest et al., 2008; Dangour et al., 2010; Nilsson, Radeborg, Salo, and Bjorck, 2012). The findings from the current survey highlight the need for more well-designed intervention trials in both young and older adults with particular emphasis on the replication of design elements such as dose, duration and cognitive measures to help shed light on the conflicting results.

The current survey showed that respondents who eat oily fish are most likely to take fish oil supplements. This result supports the inverse supplement hypothesis that respondents who already get *n*-3 PUFAs in their diet are more likely to be supplementing as

well. This finding is consistent with an Australian survey which found that fish oil consumers had a higher daily intake of finfish or seafood than non-supplement consumers despite being no different in gender, education or BMI (Grieger et al., 2013). The current survey also showed that fish oil supplement use was least likely in respondents who eat nuts and seeds. Nuts and seeds such as walnuts, flaxseeds, chia seeds and pumpkin seeds are all sources of ALA which can be converted to DHA and EPA. It is likely that this group may represent vegetarians and vegans who do not consume fish, and therefore eat nuts and seeds as a source of *n*-3 PUFAs.

The majority of fish oil users in the current survey reported taking a fish oil supplement that contained 1000 mg of fish oil equivalent to 120 mg of DHA and 180 mg of EPA per capsule. This means that a dose of at least 2000 mg of fish oil per day would be required to meet the National Heart foundation recommendations of 400-600 mg DHA and EPA. Around 26% of fish oil users would meet these recommendations, although exactly how much *n*-3 PUFAs fish oil users were also getting from their diet is unclear. With huge variation in dietary recommendations for *n*-3 PUFAs, DHA and EPA, and very limited information regarding dosing requirements for specific conditions, there is a need for further research in this area.

Contraindications between fish oil supplements and other medications particularly with anti-coagulants like warfarin can be potentially serious due to the blood thinning action of *n*-3 PUFAs. The current study showed that 62.8% of supplement users tell their GPs of their use. Over 40% of supplement users reported that they are currently taking medications prescribed by a GP and over a half (51.4%) of supplement users who take medications tell their GP. The two most common reasons for not telling their GP were that “it never came

up" or "I wasn't asked". This highlights the need for GPs to ask patients about their supplementation use.

In terms of the harmful effects of oxidised supplements, the current survey showed that only 6.8% of fish oil users reported storing their capsules in the refrigerator and 29.9% of supplement users reported purchasing their supplements at the supermarket, where supplements are typically displayed under bright florescent lights. It appears more can be done in terms of educating manufacturers, retailers and consumers in the appropriate storage conditions for this type of supplement.

Levels of the toxic mineral mercury and the sustainability of fish stocks are major concerns for consumers of fish oil supplements. Higher levels of mercury are typically found in larger predator, deep sea fish such as tuna and swordfish, but not in smaller short-lived fish such as sardines, anchovies and salmon (Mozaffarian & Rimm, 2006). Several species of fish high in *n*-3 PUFAs such as bluefin tuna are currently classified as endangered. More recently the New Zealand Institute for Plant and Food Research Plant and the Commonwealth Scientific Industrial Research Organization in Australia have begun looking at sustainable sources of *n*-3 PUFAs such as marine algae, shown to be a viable and sustainable source of DHA for vegetarians and vegans, where there are no issues with toxic mercury levels (Nichols, McManus, Krail, Sinclair, & Miller, 2014; Ryan & Symington, 2015).

There are several limitations with the current survey, largely to do with the unrepresentative sample which was mostly youngish, highly-educated females. Several questions had a high number of 'no response' which limited analysis. Respondents of the survey may have had more of an interest in supplementation, and this may be reflected in the high prevalence of supplement use.

Conclusion

Fish oil supplementation was used by 21.9% of a sample of 334 New Zealanders. Over half of these fish oil users had been taking a fish oil supplement for longer than 12 months, and it appears that those who eat oily fish are most likely to take fish oil supplements. Fish oil supplementation use has increased over the last two decades both worldwide and in NZ, yet questions remain about the role of DHA and EPA in brain health, what exactly is a 'sufficient' daily dose and which health conditions show the most improvement from taking *n*-3 PUFAs. Safety issues related to manufacturing and storage conditions point to an urgency in answering these questions.

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CHAPTER 5

DHA SUPPLEMENT TRIALS IN OLDER ADULTS

This chapter will review 23 randomised controlled trials (RCTs) that have investigated the cognitive effects of DHA supplements in older adults. For the purpose of this thesis, this review was limited to RCTs that used a capsulated DHA supplement, and which included participants within an age range or a mean age extending past the age of 60 to allow comparison with the current RCT. Details of the design and cognitive outcomes of each trial are summarised in Tables 6, 7 and 8. The chapter ends with a summary of all 23 RCTs.

5.1 Cognitively healthy older adults

Four large-scale intervention trials found no effect from DHA fish oil supplementation on cognitive performance in cognitively healthy older adults (see Table 6, studies 1-4). The first trial by van de Rest et al. (2008a) compared a high and low dose fish oil supplement with a placebo in 299 older adults, where the low dose was comparable to the Netherlands recommended intake of two fish portions per week with one oily and one non-oily fish. No main significant differences were found between the three groups on any of the cognitive tests, although there was a treatment effect in the attention domain (Digit Span Forwards Task) for APOE ε4 carriers indicating that ε4 carriers in both the low (p = .03) and high (p = .04) fish oil groups showed a significant improvement. There were no significant

differences between the three groups on four depression scales including the short version of the Geriatric Depression Scale (GDS-15) (van de Rest et al., 2008b). In the second large (*n* = 728) trial by Dangour et al. (2010), a specific dose of *n*-3 PUFAs was used as it could be achieved by eating 250 g of oily fish per week (equivalent to 1.75 portions), and was based on UK recommendations for oily fish consumption. The results showed no differences between the fish oil group and placebo group in the cognitive measures over the two years of supplementation.

In the third trial by Chew et al. (2015), 3024 older adults with drusens (yellow deposits under the eye) or late age-related macular degeneration (AMD) were allocated to one of four groups: DHA/EPA fish oil group, lutein/zeaxanthin group, DHA/EPA and lutein/zeaxanthin mix group and placebo group. Lutein is a carotenoid, typically found in red, orange and yellow fruits and vegetables that have anti-oxidant actions, particularly related to age-related decline, and zeaxanthin is one of the main carotenoids found within the retina (Craft, Haitema, Garnett, Fitch, & Dorey 2004). At the 5-year mark, neither the fish oil nor the lutein/zeaxanthin treatments had a statistically significant effect on cognitive function. The results showed that lower cognitive function at baseline was associated with increasing age, lower education level, and the male sex, as well as hypertension and other cardiovascular diseases including stroke. The fourth trial by Danthiir et al. (2018) used a high dose DHA fish oil supplement (1720 mg DHA + 600 mg EPA per day) in a final sample of 390 older adults for 18 months. Again, there were no main significant differences between the fish and placebo groups on the cognitive measures, although a negative treatment effect was found for simple and choice reaction time ($p < .01$) and psychomotor speed ($p < .01$) in men, and for retrieval fluency ($p = .01$) in women. APOE $\varepsilon 4$ carriers the fish oil group showed

a significant improvement on reasoning (p = .02), whereas non-ε4 carriers performed worse on simple and choice reaction time (p = .01).

Two smaller trials also found no treatment effect on cognitive performance in cognitively healthy older adults (see Table 6, studies 5 & 6). Stough et al. (2012) gave healthy older adults fish oil or a placebo for 90 days. The results showed no differences between the two groups in the cognitive measures as measured by a cognitive drug research computerised assessment system, including 11 tasks used to calculate five factor scores – speed of attention, speed of memory, accuracy of attention, secondary memory and working memory. The authors found that for participants with corrected vision, the fish oil group had significantly improved right eye visual acuity at the 90-day mark. Pase et al. (2015) conducted a 16-week trial combining fish oil supplementation with multi-vitamin (MV) supplements – high dose fish oil + MV, low dose fish oil + MV, high dose fish oil, placebo fish oil and placebo MV groups. There were no cognitive differences between the groups at the 16-week mark, although there was an association between the *n*-3:*n*-6 ratio and faster spatial working memory (r = -.17, p < .01). Compared to the placebo fish oil/placebo MV groups, there were no changes in DHA levels in the three groups. The study found that levels of *n*-6 PUFAs decreased in the high fish oil group, and that decreases in *n*-6 PUFA were associated with decreases in brachial (r = .23, p < .01) and aortic pulse (r = .21, p < .01) pressure, and that increases in DHA were associated with decreases in diastolic aortic pressure (r = -.18, p < .01).

Several smaller trials have found positive results in cognitively healthy older adults (see Table 6, studies 7-11). Johnson et al. (2008) conducted a 4-month trial where participants were divided into four groups: DHA, lutein + zeaxanthin, DHA + lutein and a placebo. Lutein was included to investigate the effects of fruit and vegetable consumption

on cognition, without and in combination with *n*-3 PUFAs. At the 4-month mark women in the DHA ($p = .03$, $d = 0.68$), lutein ($p < .01$, $d = 0.79$) and DHA + lutein groups ($p < .01$, $d = 1.54$) scored better on a verbal fluency task compared to baseline. The DHA + lutein group showed a faster rate of learning on the shopping list task ($p = .03$, $d = 0.74$), recalled more items in a memory apartment test ($p = .02$, $d = 0.58$) and were more efficient at learning a word list ($p = .07$, $d = 0.78$) after 4-months of supplementation. After adjusting for age, higher DHA serum levels were associated with higher verbal fluency scores ($r = .24$, $p = .04$). Nilsson et al. (2012) conducted a 5-week crossover design trial in order to relate cognitive outcomes to cardiovascular parameters. The trial found significant treatment effects on working memory at 60-minutes ($p = .04$, $d = 0.26$), working memory test with nonsensical sentences ($p = .01$, $d = 0.34$) and working memory at 60 + 160-minutes ($p = .02$, $d = 0.31$). The study also showed that systolic blood pressure ($p = .04$, $d = 0.46$) and serum triglycerides ($p = .05$, $d = 0.59$) were significantly reduced after fish oil supplementation compared to after the placebo period, and that systolic blood pressure was inversely related to cognitive performance.

Witte et al. (2013) found a fish oil treatment effect using an executive composite score (included z-scores of fluency tests, Trail Making Test versions B-A and Stroop), compared to the placebo group ($p = .01$, $d = 0.75$). The fish oil supplement group showed a significant increase in EPA proportions ($p < .01$, $d = 1.49$), and the results showed that letter fluency scores were correlated with these increases in EPA ($r = .46$, $p = .01$). There was a trend for memory consolidation according to a change in the omega-3 index, with the fish oil group showing more improvement than the placebo group ($p = .09$). The fish oil supplement resulted in significant increases in white matter structural integrity and increases in grey matter regional volume in the left hippocampus, precuneus, superior temporal, inferior

parietal and postcentral gyri, and right middle temporal gyrus. The authors also noted that improvements in executive functions in the fish oil group were associated with relative increases in brain-derived neuro-trophic factor (BDNF) (r = .46, p = .02), and that the fish oil group had a significant decrease in both diastolic blood pressure (p = .05, d = 0.34) and fasting triacylglycerides (p = .01, d = 0.39).

Strike, Carlisle, Gibson, and Dyall (2015) tested a DHA + EPA + *Ginkgo biloba* supplement and found significantly shorter psychomotor latencies (information processing speed)(p = .04, η_p^2 = .13), significantly more words remembered (verbal recognition memory)(p = .03, η_p^2 = .15) and an improvement in habitual walking speed (a measure of overall health and function) in the treatment group (p = .03, η_p^2 = .14). There were positive associations between DHA levels and habitual walking speed (r = .46, p < .05), fast walking speed (r = .55, p < .01) and vertical height jump (r = .49, p < .01). Külzow et al. (2016) used a visuospatial object location memory task (LOCATO) to assess cognitive performance with both learning and recall components. LOCATO was designed to be a meaningful and ecological assessment for older adults, as it assesses the ability to remember the location of objects, an ability that declines with age. Recall performance was significantly better in the fish oil group (p = .05, η_p^2 = .09) than the placebo group after controlling for dietary habits and APOE ε4 carrier status (p = .01, η_p^2 = .17) but not in the learning component of the LOCATO or in a German version of the RAVLT.

The results from the DHA supplement trials in cognitively healthy older adults are conflicting. The four large RCTs found no treatment effects except for a positive effect in attention and reasoning for APOE ε4 carriers. However, several of the smaller studies found positive treatment effects on memory (recall and working memory), and positive associations between increases in EPA and DHA and improvements in cognitive measures.

Table 6. Summary of fish oil trials in cognitively healthy older adults

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive Results	Sampling tissue/change
1.	van de Rest et al. 2008	Netherlands	26-weeks	Rx1: 96/1% Rx2: 100/0% Pb: 106/2.8% Total: 299	66-74 *M*=70	Rx1: 847 DHA + 1093 EPA Rx2: 176 DHA + 226 EPA 6 capsules/day	High-oleic acid sunflower oil	No significant differences, although there was a treatment effect in APOE ε4 carriers and in men in the digit span forwards task	Plasma – Fatty acid profiles and APOE genotyping. EPA+DHA increased by 236% in Rx1 and 51% in Rx2
2.	Dangour et al. 2010	UK	24-months	Rx: 434/13.4% Pb: 433/14.1% Total: 748	70-79 *M*=75	Rx: 500 DHA + 200 EPA 2 capsules/day	Olive oil	No significant differences on any of the cognitive measures	Serum – Fatty acid profiles at 24-months. Rx showed higher DHA and EPA, and lower ARA
3.	Chew et al. 2015	USA	5-years	Rx1: 1069/0.6% Rx2: 1044/0.6% Rx3: 1079/0.9% Pb: 1012/0.5% Total: 3024	50-85 *M*=74	Rx1: 350 DHA + 650 EPA Rx2: 10 lutein + 2 zeaxanthin Rx3: 350 DHA + 650 EPA + 10 lutein + 2 zeaxanthin	Not specified	Fish oil showed no statistically significant effect on cognitive function in older adults with intermediate age-related macular degeneration	Serum
4.	Danthiir et al. 2018	Australia	18-months	Rx: 203/4.4% Pb: 200/2% Total: 390	65-90 *M*=73	Rx: 1720 DHA + 600 EPA 4 capsules/day	Olive oil + 10 mg fish oil equivalent to 1.2 mg DHA + 1.8 mg EPA	No significant differences on the cognitive measures, except for a positive effect for APOE ε4 carriers in reasoning	RBC – Fatty acid levels and APOE. EPA levels increased by 212.4 % and DHA increased by 84.9 % in Rx group

Note. APOE = Apolipoprotein genotype, ARA = Arachidonic acid, DHA = Docosahexaenoic acid, EPA = Eicosapentaenoic acid, *M* = Mean, Pb = Placebo, RBC = Red blood cell, Rx = Treatment

Table 6. (continued)

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive results	Sampling tissue/change
5.	Stough et al. 2012	Australia	90-days	Rx: 38 Pb: 36 No attrition information	45-77 *M*=57	Rx: 252 DHA + 60 EPA 1 capsule/day	Soybean oil	No significant cognitive differences using the Cognitive drug research computerized assessment system	Plasma – Levels of Phospholipid DHA. Rx showed 1.79% raised plasma phospholipid DHA levels
6.	Pase et al. 2015	Australia	16-weeks	Rx1: 43/11.6% Rx2: 39/7.6% Rx3: 41/7.3% Pb: 37/13.5% Total: 144	50-70 *M*=59	Rx1: 240 DHA+ 240 EPA + MV 3 capsules/day Rx2: 480 DHA+ 480 EPA + MV 6 capsules/day Rx3: 480 DHA+ 480 EPA 6 capsules/day	High-oleic acid sunflower oil & Placebo MV of carrot and riboflavin powder	No significant cognitive differences using a computerised cognitive battery, although increases in the *n*-3:6 ratio were associated with faster spatial working memory response times	RBC – Fatty acid levels. EPA increased by 96% in Rx2, 57% in Rx3 but not in Rx1. No changes in DHA levels in all three Rx groups
7.	Johnson et al. 2008	USA	4-month	57/86% Total: 49 Rx1: 14 Rx2: 11 Rx3: 14 Pb: 10	60-80 *M*=68	Rx1: 800 DHA Rx2: 12 lutein Rx3: 800 DHA+ 12 lutein 1 capsule/day	No placebo information	DHA, lutein and DHA+lutein groups improved on verbal fluency, and DHA+lutein group showed improved rate of learning and recall compared to placebo	Serum – DHA and lutein levels

Note. MV= Multi-vitamin

Table 6. (continued)

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive results	Sampling tissue/change
8.	Nilsson et al. 2012	Sweden	5-weeks (plus a 5-week placebo and a 5-week wash-out period)	Rx1: 22/9.1% Rx2: 22/18.2% Total: 38	51-72 *M*=63	Rx1: 1050 DHA + 1500 EPA →Pb Rx2: Pb →1050 DHA + 1500 EPA 5 caps/day	Di-calcium phosphate + cellulose + magnesium salts of fatty acids 2 caps/day	Significant differences between the fish oil and placebo intervention periods in several working memory measurements	Serum – free fatty acids
9.	Witte et al. 2013	Germany	26-weeks	Rx: 40/20% Pb: 40/17.5% Total: 65	50-75 *M*=64	Rx: 880 DHA + 1320 EPA 4 capsules/day	Sunflower oil	Improved executive score (included fluency tests, TMT versions B-A and Stroop) in fish oil group compared to placebo	RBC – fatty acid levels, BDNF. Increase in EPA
10.	Strike et al. 2015	UK	6-months	Rx: 15/0% Pb: 13/7.7% Total: 27	60-84 *M*=67	Rx: 1000 DHA + 160 EPA + 240 *Ginkgo biloba* + phosphatidylserine + Vitamin E + folic acid + Vitamin B12 4 capsules/day	Iso-calorific oil	Shorter motor latencies and more words remembered in treatment group compared to placebo group	Whole blood – fatty acid levels. Rx showed a 17% increase in DHA, a 71% increase in EPA and an 18% decrease in ARA
11.	Külzow et al. 2016	Germany	26-weeks	Rx: 40/45% Pb: 40/45% Total: 44	50-75 *M*=62	Rx: 880 DHA + 1320 EPA 4 capsules/day	Sunflower oil	Recall of object location was significantly better in the fish oil group than the placebo group	RBC/DNA – total fatty acid levels + APOE genotyping. Significant increase in the omega-3 index in Rx group

Note. BDNF = Brain-derived neuro-trophic factor, DNA = Deoxyribonucleic acid, TMT = Trail Making Test

5.2 Older adults with cognitive impairment and dementia

In older adults with dementia, the results from *n*-3 PUFA trials are also very mixed. The first of these trials by Quinn et al. (2010) gave participants with mild to moderate Alzheimer's disease (AD) an algal derived DHA supplement for 18 months (contained no EPA) (see Table 7, study 12). DHA supplementation did not slow the rate cognitive or functional decline as measured by changes in the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) and the Clinical Dementia Rating (CDR) sum of boxes. There were also no differences in the change in total brain volume using MRI between the two groups. Quinn et al. found a significantly lower decline in mean change in the ADAS-cog for APOE non-ε4 carriers in the fish oil group (p = .03, d = 0.76) than for non-ε4 carriers in the placebo group.

More promising results have been found in milder forms of dementia (see Table 7, studies 13 & 14). Freund-Levi et al. (2006) found no differences between two groups of older adults with AD using the MMSE and ADAS-cog, although there was a significant difference in the change in mean MMSE scores in a subgroup of patients with a MMSE score > 27 points (classified as 'mild AD') (p < .01, d = 0.68) in favour of the fish oil group. There were also significant treatment effects for two MMSE sub-items, delayed word recall (p < .01, d = 0.31) and attention (p < .01, d = 0.58). Using the Neuropsychiatric Inventory, Freund-Levi et al. (2008) found significant differences in hallucination (p = .04) scores changes in favour of the fish oil group, in irritation (p = .01) scores changes in favour of the placebo and a significant positive treatment effect in the agitation domain scores for APOE ε4 carriers (p = .01). Using the Montgomery Asberg Depression Scale the authors also found that non-ε4 carriers in the fish oil group showed a significant positive effect on scores (p = .01) compared to non-ε4 carriers in the placebo group. A pilot study by Terano et al. (1999) found that in a group of older adults with dementia from thrombotic cerebrovascular disorders, DHA group scores improved on the Hasegawa's Dementia

Rating Scale (HDS-R) after three months ($p < .05$, $d = 0.57$) and six months ($p < .05$, $d = 0.48$) and on the MMSE after six months ($p < .05$, $d = 0.39$) but not after 12 months. The authors found that red blood cell (RBC) deformability significantly improved in the DHA group ($p < .01$), and that DHA:ARA ratio was positively correlated with scores on the HDS-R ($r = .44$, $p < .05$).

Three clinical trials included both older adults with dementia and older adults with mild cognitive deficits, and two found a significant cognitive effect but only in the mild cognitively impaired older adults (see Table 7, studies 15-17). The first trial by Kotani et al. (2006) used a Japanese version of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in older adults with MCI, AD and organic brain lesions. Kotani et al. found that older adults with MCI showed significant improvements in both immediate memory ($p < .01$) and attention ($p < .05$) from a DHA + ARA supplement compared to the placebo, and that the participants with organic brain lesions showed improvement from the baseline scores in immediate ($p < .01$) and delayed memory ($p < .01$) from the DHA + ARA supplement.

The second trial by Chiu et al. (2008) with AD and MCI participants found a significant improvement in disease severity as measured by the Clinician's Interview-Based Impression of Change scale in those who took the fish oil supplement ($p = .01$). After adjusting for age, gender and education, MCI participants in the fish oil group showed greater improvement in the ADAS-cog than the placebo group ($p = .03$), whereas the change in ADAS-cog scores in AD participants was the same for the fish oil and placebo groups. The trial also found that better ADAS-cog scores were associated with higher levels of EPA ($p < .01$). The third trial by Phillips, Childs, Calder, and Rogers (2015) involved older adults with Cognitive impairment no dementia (CIND) and AD, and found no significant treatment effects for any of the cognitive or mood measures, although there was a trend for improved visual memory ($p = .09$) from the Rivermead Behavioural Memory Test in favour of the *n*-3 PUFA group.

Two trials have specifically focused on older adults with subjective memory complaints and MMSE scores below 26 (see Table 7, studies 18 & 19). The first trial by Yurko-Mauro et al. (2010) used an algal supplement in older adults with age-related cognitive decline (ARCD). Participants were further excluded if they had a raw score of ≥ 1 SD below the mean for younger adults in the logical memory subtest from the Wechsler Memory Scale (WMS-III). The DHA group had significantly fewer errors on the Cambridge Neuropsychological Test Automated Battery Paired Associate Learning (PAL) Test (p = .03), and improved immediate (p = .02) and delayed verbal memory (p = .01) compared to participants in the placebo group at the 24-week mark. Plasma DHA levels significantly increased in the DHA group (p < .01) and were correlated with improved PAL scores (r = -.11, p < .02). The DHA group also showed a significant decrease in heart rate (p < .03) at the 24-week mark.

The second trial by Vakhapova, Cohen, Richter, Herzog, and Korczyn (2010) investigated the effects of a phosphatidylserine (PS) and DHA combination (PS-DHA) vs. a placebo in non-demented elderly participants with memory complaints for 15-weeks. The study showed that the PS-DHA group had significantly improved immediate recall in the RAVLT (p = .04) at the 15-week mark compared to the placebo group. The authors found that participants in PS-DHA group with a higher cognitive status at baseline (n =78) showed the most improvement in RAVLT immediate recall (p = .01), total learning (p < .01) and delayed recall (p = .04).

The DHA supplement trials in older adults with cognitive impairments and dementia have also found conflicting results, but with more positive results in milder forms of dementia.

Table 7. Summary of fish oil trials in older adults with cognitive impairment and dementia

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive results	Sampling tissue/change
12.	Quinn et al. 2010	USA	18-months	Rx: 238/28.1% Pb: 164/24.4% Total: 295	*M*=76	Rx: Algal – approx. equiv. to 1000 DHA 2 capsules/day	Either corn or soya oil	DHA supplementation did not slow the rate of decline as measured by changes in the ADAS-cog and CDR sum of boxes	Plasma, CSF – phospholipid fatty acid levels, APOE. DHA levels increased by 207% in the Rx group
13.	Freund-Levi et al. 2006/8	Sweden	6-months (plus a 6-month period of Rx fish oil)	Rx: 103/13.6% Pb: 101/15.8% Total: 174	*M*=73	Rx: 1720 DHA + 600 EPA 4 capsules/day	4000 mg of corn oil equivalent to 2400 mg of LA	No differences were found between the two groups using the ADAS-cog; fish oil group with a MMSE score > 27 showed significantly different change in MMSE scores from baseline to 6-months	Serum – fatty acid levels, APOE. Rx group showed a 2.4-fold increase in DHA and a 3.6-fold increase in EPA
14.	Terano et al. 1999	Japan	12-months	Rx: 10 Pb: 10 Total: 20	*M*=83	Rx: 720 DHA 6 capsules/day	Not specified	DHA group scores improved on the HDS-R and MMSE compared to the placebo after 3 and 6-months	Serum – fatty acid levels. In the RX group DHA levels increased by 79% and EPA by 70%
15.	Kotani et al. 2006	Japan	90-days	MCI-Rx: 12 MCI-Pb: 9 AD-Rx: 8 OBL-Rx: 10 Total: 39	*M*=64	Rx: 240 DHA + 240 ARA 6 capsules/day	240 mg of Olive oil	MCI-Rx showed significant improvements in immediate memory and attention; OBL showed significant improvement in immediate and delayed memory	Serum – fatty acid levels. No serum changes were found

Note. ADAS-cog = Cognitive subscale of the Alzheimer's Disease Assessment Scale, AD = Alzheimer's disease, CDR = Clinical Dementia Rating, CSF = Cerebrospinal fluid, HDS-R = Hasegawa's Dementia rating scale, LA = Linoleic acid, OBL = Organic brain lesions

Table 7. (continued)

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive results	Sampling tissue/change
16.	Chiu et al. 2008	Taiwan	24-weeks	MCI: 23 AD: 23 Rx: 24/29.1% Pb: 22/45.5% Total: 29	70-82 *M*=75	Rx: 720 DHA + 1080 EPA 3 capsules/day	Olive oil 3 caps/day	MCI-Rx group showed a significant improvement in ADAS-cog score than placebo group; no change in ADAS-cog scores in AD-Rx	RBC and plasma – fatty acid levels
17.	Phillips et al. 2015	UK	4-months	CIND:57 AD:19 Rx:37/2.7% Pb:39/7.7% Total: 72	*M*=71	Rx: 625 DHA + 600 EPA 2 capsules/day	Olive oil	No significant treatment effects were found for the cognitive or mood measures	Plasma – phospholipid fatty acid levels. DHA levels increased by 38.1% and EPA by 137.5% in Rx group
18.	Yurko-Mauro et al. 2010	USA	24-weeks	Rx: 242/9.5% Pb: 243/10.3 Total: 437	*M*=70	Rx: 900 DHA 3 capsules/day	50% corn oil + 50% soy oil	DHA group had significantly fewer CANTAB PAL errors as well as improved immediate and delayed verbal memory scores compared to the placebo group	Plasma – phospholipid fatty acid levels. Plasma DHA levels significantly increased in the Rx group
19.	Vakhapova et al. 2010	Israel	15-weeks	Rx: 79/24% Pb: 78/20.5% Total: 122	*M*=73	Rx: 79 as 3:1 DHA:EPA + 300 PS 3 capsules/day	Cellulose	PS-DHA group had significantly improved immediate recall; Ps with higher cognitive status at baseline showed the most improvement in immediate recall, total learning and delayed recall	

Note. CANTAB PAL = Cambridge Neuropsychological Test Automated Battery Paired Associate Learning, CIND = Cognitive impairment no dementia, Ps = Participants, PS = Phosphatidylserine

5.3 Older adults with Mild Cognitive Impairment

The first trial to specifically investigate the cognitive effects of a DHA supplement in older adults with MCI was by Sinn et al. (2012) who compared an EPA group, a DHA group and a placebo group (see Table 8, study 20). MCI was defined as a subjective complaint of memory loss with otherwise normal daily functioning and cognitive assessment scores of ≤ 1.5 SD below the population age mean. Scores improved significantly on the GDS in both the DHA (p = .01) and EPA (p = .04) groups compared to the placebo group. There was also a significant improvement in Initial Letter Fluency for the DHA group (p = .04), and an improvement in the DHA group in the Digit Span Backwards Test, though not significant *(*p = .10). Increased RBC levels of DHA and EPA were associated with improvements on the GDS (r = .39, p = .02), and increased RBC levels of DHA were associated with improved physical health (r = .39, p < .05) and physical functioning (r = .37, p < .05) from the 36-Item Short Form Health Survey Questionnaire (SF-36), in particular reduced bodily pain (r = .35, p < .05). A reduced ARA:EPA ratio was associated with improvements on the GDS (r = .34, p < .05) as well as improvements in both mental health (r = .50, p < .01) and vitality (r = .34, p < .05) scores from the SF-36.

In the same year, Rondanelli et al. (2012) investigated a combination supplement containing DHA and EPA (see Table 8, study 21). Of note is that the supplement and placebo groups differed significantly at baseline on the GDS *(Msupplement* = 21.5, *Mplacebo* = 12.7, p = .00), with a trend for years of education *(Msupplement* =5.4, *Mplacebo* = 7.3, p = .07). MCI was defined as a memory complaint referred by the participant or a family member, a cognitive impairment in more than one domain (executive function, memory, language, or visuospatial), normal functional activities and not having dementia. At the 12-week mark the supplement group showed a significant treatment effect on the MMSE (p < .01) and a trend in semantic fluency (p = .06). A significant improvement (p = .01) in the supplement group using

the Mini Nutritional Assessment tool (includes anthropometric measures, medication use and mobility) was also found. This trial also used an olfactory sensitivity assessment called the 12-item Sniffin' Sticks Screening Test based on research which suggests it can be a useful measure in predicting the progression from amnesic MCI to AD (Eibenstein et al., 2005). The results found that the supplement groups' ($p < .01$) scores significantly improved on the Sniffin' Sticks Screening Test compared to the placebo group.

The following year Lee, Shahar, Chin, and Yusoff (2013) published a fish trial in older adults with MCI (see Table 8, study 22). MCI was defined as the presence of a memory complaint (either self-reported or by family members or caregivers), preserved normal cognitive function (assessed by the MMSE), intact activities of daily living (1.5 SD from mean), not demented (1 SD from the norm on the Dementia Rating Scale), not depressed (4 points on the GDS short version) and abnormal cognitive impairments for age (>1 SD from the norm). At the end of the 12-month trial and after controlling for age, education and baseline blood pressure, the DHA + EPA treatment group showed a significant improvement in three of the four memory tasks – Digit Span Backwards ($p < .01$), Visual Reproduction I ($p < .05$) and RAVLT delayed recall ($p < 0.05$), which test short-term memory, working memory, immediate verbal memory and delayed recall. Scores did not significantly differ between the two groups on the GDS at either the 6-month or 12-month mark.

A fourth MCI trial was published by Yacong et al. (2017) and was designed to test an EPA:DHA ratio of 3:2, and to investigate the link between cognition and inflammation (see Table 8, study 23). MCI was defined based on the criteria by Petersen et al. (1999) with the inclusion criteria of a memory or cognitive disorder for at least three months and an MMSE score of 24 to 26 for individuals who had >6 years of education, 20 to 22 for <6 years, and 17 to 19 for participants without education. The results of the trial showed that after six months

the *n*-3 PUFA group had a significant improvement in the total Basic Cognitive Aptitude Test score *(p* < .01), perceptual speed *(p* < .01), space imagery efficiency *(p* < .01), working memory *(p* < .05), with a trend for mental arithmetic efficiency *(p* = .06) but not recognition memory *(p* = .58). The authors found that the *n*-3 PUFA supplement resulted in significant decreases in interleukin IL-6, tumour necrosis factor TNF-α, sPLA2 *(p* < .05), all of which are signals of inflammation.

The results from the DHA supplement trials in older adults with MCI have been very promising. Overall, these four trials have found positive effects for fluency, short-term memory, working memory, immediate verbal memory, recognition and delayed recall as well as depression scores.

Table 8. Summary of fish oil trials in Mild Cognitive Impairment (MCI)

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive Results	Sampling tissue/change
20.	Sinn et al. 2012	Australia	6-month	Rx1: 18/27.7% Rx2: 18/11.1% Pb: 18/38.8% Total: 40	≥ 65 *M*=74	Rx1: 160 DHA + 1670 EPA Rx2: 1550 DHA + 400 EPA 4 capsules/day	Safflower oil equivalent to 2200 mg of LA per day	A significant improvement in letter fluency for the DHA group compared to the ALA group; scores improved significantly on the GDS in both the DHA and EPA groups; increased RBC levels of DHA were associated with improved self-reported physical health and physical functioning	RBC – fatty acid levels. Over a 4-fold increase in EPA in Rx1 and a 2-fold increase in both EPA and DHA in Rx2
21.	Rondanelli et al. 2012	Italy	12-weeks	Rx: 11/0% Pb: 14/0% Total: 25	≥ 70 *M*=86	Rx: 720 DHA + 286 EPA + 160 PLP + 95 TRP + 5 MEL 2 capsules/day	Non-fish oils	Found a significant treatment effect in the MMSE, MNA and in olfactory sensitivity in the supplement group, as well as a trend in semantic fluency in favour of the supplement group	Fasting blood samples

Note. GDS = Geriatric Depression Scale, PLP = Phospholipids, MEL = Melatonin, MMSE = Mini-Mental State Examination, MNA = Mini Nutritional Assessment, TRP = Tryptophan

Table 8. (continued)

#	Author/Year	Country	Duration	Sample size/ Attrition rate	Age	Intervention (mg/day)	Placebo	Cognitive Results	Sampling tissue/change
22.	Lee et al. 2013	Malaysia	12-months	Rx: 18/5.5% Pb: 18/0% Total: 35	≥ 60 *M*=65	Rx: 1290 DHA + 450 EPA 3 capsules/day	Corn oil equivalent to 1800 mg of LA per day	The DHA+EPA group showed a significant improvement in short-term memory, working memory, immediate verbal memory and delayed recall after controlling for age, education and baseline blood pressure	Plasma – fatty acid levels. Plasma levels of DHA and EPA increased by 83.7% and 121.6% in Rx group
23.	Yacong et al. 2017	China	6-months	Rx: 44/27% Pb: 42/23.8% Total: 64	≥ 60 *M*=71	Rx: 480 DHA + 720 EPA 4 capsules/day	Olive oil equivalent to 2200 mg of oleic acid 4 caps/day	DHA+EPA group had a significant improvement in total BCAT score, as well as sub-items perceptual speed, space imagery efficiency and working memory; DHA+EPA group showed significant decreases in several inflammatory signals	Plasma – phosphatidyl-choline fatty acid levels, cytokines, enzymes. Increased levels of plasma DHA and EPA

Note. BCAT = Basic Cognitive Aptitude Test

5.4 Meta-analysis of DHA trials in older adults

In 2012, Mazereeuw, Lanctot, Chau, Swardfager, and Herrmann performed a meta-analysis of three *n*-3 PUFAs trials with cognitively healthy older adults (studies 1, 2 & 7) and seven trials involving older adults with memory complaints, CIND, MCI and dementia (studies 12, 13, 15, 16, 18, 19 & 20). The inclusion criteria for the meta-analysis required that studies were RCTs with measurable neuropsychological results, used a supplement form of *n*-3 PUFAs, and included participants aged 50 years and older without psychiatric comorbidity. Results from the neuropsychological tests were classified into five cognitive domains – immediate recall, delayed recall, recognition, working memory/executive function and attention/processing speed, in addition to composite memory (pooled memory outcomes) and global cognitive functioning as measured by MMSE and ADAS-cog.

When all participants were combined, the results of the meta-analysis found that *n*-3 PUFAs did not provide a significant benefit in any of the cognitive domains. However, Mazereeuw et al. (2012) found a significant effect in the subgroup of older adults with CIND in the cognitive domains of immediate recall *(p* = .03, g = 0.16) and attention/processing speed *(p* = .04, g = 0.32). There was also a significant treatment effect in delayed verbal recall *(p* = .02, g = 0.30) in a subgroup analysis of high-functioning CIND and AD participants (MMSE ≥ 26). The meta-analysis also compared safety and tolerability of *n*-3 PUFA vs. placebo supplements and found no differences in drop-outs and adverse effects, implying that *n*-3 PUFA supplements are safe and well tolerated. The authors noted that dose, duration and baseline MMSE scores were not related to treatment effect in any of the cognitive domains. In the case of dose, the authors suggest this finding indicates that *n*-3 PUFA supplementation may work by surpassing a DHA threshold level rather than by a causing a big change in DHA levels. With no standard reference ranges or testing procedures

for assessing *n*-3 PUFAs, DHA or EPA levels, exactly what a 'low' or a 'high' baseline level is or where this DHA threshold level may sit, is not clear.

5.4 Summary of DHA trials

The difficult task for researchers in the area of DHA and *n*-3 PUFA interventional trials is trying to make sense of the conflicting results. The results of the meta-analysis suggest that *n*-3 PUFAs may provide the most benefit for older adults with mild forms of cognitive impairment, specifically in the areas of verbal recall and attention. Defining participant groups is a major challenge within this area of research, particularly in milder forms of cognitive impairment. It is comparatively easy to define and measure dementia, although to show that an intervention prevents or improves dementia is difficult as any improvements in AD may be overcome by the progression of the disease. As a result, the rate of decline is often used as an outcome measure in AD as opposed to an improvement in cognition. The need for universal measures of both global and specific cognitive domains is controversial, and results in most clinical trials opting to use the MMSE. Both van de Rest et al. (2008) and Dangour et al. (2010) used the MMSE to assess whether participants were cognitively healthy at baseline, where van de Rest et al. used the cut-off of scores above 23, and Dangour et al. scores above 25. Several of these trials relied on global cognitive measures or memory tasks only, even though it is quite clear that the best type of cognitive assessment involves a battery of memory along with executive functioning and language tasks.

The main issue with the MMSE as a cognitive measure is that, especially in high functioning or highly educated older adults, it often results in a ceiling effect. In contrast, measures such as word lists, working memory and attention tasks such as digit spans, and

semantic fluency tasks show no ceiling, and in some cases, no practice effects after three months making them particularly useful for longer trials (e.g., Bartels et al., 2010). van de Rest et al. chose to select participants who were 'cognitively still intact' by way of an MMSE score above 23 based on the idea that it may be 'too late' for fish oil supplementation to counteract the process of decline in participants who have mild to severe cognitive impairment. The authors also noted though that the participants in their study may have been 'too good' for fish oil supplementation to be effective. Lee et al. (2013) commented that the discrepancies in the results from *n*-3 PUFAs trials in older adults are mainly due to the extent of the cognitive impairment. Lee et al. further stated the participants in their trial were mildly impaired, with the results showing participants in their trial had a MMSE >25. Of the four MCI trials, Sinn et al. (2012) was the only trial to use a cut-off of 1.5 SDs for the cognitive deficit and Yacong et al. (2017) was the only trial to take into account 'years of education', but only in regard to the MMSE cut-off point. Research indicates that measuring pre-morbid functioning is more reliable than using years of education, as the quantity of education is not always an indication of the quality, as years of education can be influenced by many 'non-cognitive factors' such as gender, income and ethnicity (Ahl et al., 2013). There are major difficulties involved in recruiting participants, particularly older adults with mild cognitive impairments, which can result in the inclusion of a wide range of 'mildly' impaired participants.

Two of the trials reviewed in this chapter were conducted in Germany using the same fish oil supplement (same brand and supplier), with the same daily dose (880 mg of DHA and 1320 mg of EPA) and with the same duration (26 weeks), but with different measures (Witte et al., 2013; Külzow et al., 2016). Both trials were focused on cognitively healthy older adults, used a MMSE score of > 26 as the cut-off, and both trials used the

omega-3 index as a measure of change in *n*-3 PUFA levels. The trial by Witte et al. used a cognitive battery to assess all cognitive domains – Trail Making Test A & B, a verbal fluency test, a version of the Stroop Test and Digit Span Forwards and Backwards, whereas Külzow et al. used a visuospatial object location memory task called LOCATO containing a learning and a recall component. Witte et al. found that the fish oil group significantly improved on the executive composite score using the fluency, Trial Making versions B-A and Stroop Test, but only a trend for memory consolidation when the analysis focused on participants who responded best to the intervention, as measured by a change in the omega-3 index. Külzow et al. found that recall performance, but not learning, was significantly better in the fish oil group after controlling for dietary habits and APOE status. Interestingly, Witte et al. measured grey matter changes and found significant increases in left hippocampus volume in the fish oil group, and Külzow et al. noted that object location memory is dependent on proper hippocampal functioning. It is likely that the fish oil groups in both trials had changes in hippocampal activity, and that the different tests tapped into this activity in different ways. This highlights the importance of selecting measures with appropriate validity and sensitivity, including both memory and executive functioning measures, as well as commonly used measures to allow for comparison across trials.

When reporting the results of clinical trials, there is a tendency to focus on the significant differences between the *n*-3 PUFA and placebo groups, but not the negative results or the significant differences in favour of the placebo. The meta-analysis by Mazereeuw et al. (2012) found that *n*-3 PUFAs improved verbal recall and attention in older adults with CIND, and an examination of the results for cognitively healthy older adults also showed that improvements tended to be in verbal recall and attention, as well as in verbal fluency and working memory. An examination of the negative treatment results showed

that they were in visuo-spatial/constructional tasks, speed and motor tasks, executive function tasks like spatial planning, block design and clock drawing, and most language tasks except for verbal fluency. Also, despite the randomisation element of RCTs, baseline differences between treatment and placebo groups do occur, as do differences between those who complete the trial and those who drop-out. For example, in the trial by Dangour et al. (2010) the authors noted that drop-outs had a lower baseline level of cognitive function as measured by the California Verbal Learning Test. The importance of reporting all results, and in particular how the differences were analysed, has recently been highlighted by the 2018 American Psychological Association guidelines (Appelbaum et al., 2018).

Out of the 23 trials reviewed in this chapter, 15 trials used an *n*-3 PUFA supplement that contained only fish oil as the main ingredient, two trials used supplements containing fish oil in combination with lutein and zeaxanthin, one trial used fish oil in combination with a multi-vitamin/mineral complex, one used phosphatidylserine in combination with DHA, two used algal supplements, and two trials used combination supplements – one with the addition of the herbal extract *Ginkgo biloba* and one with the addition of phospholipids + 95 mg of tryptophan + 5 mg of melatonin. The reason for using an algal supplement or a co-factor such as a phospholipid/ phosphatidylserine or additional nutrients/extracts such as lutein or *Ginkgo biloba* is that they have the action of enhancing the biosynthesis of *n*-3 PUFAs, making DHA more bio-available, or the ingredient has been independently shown to reduce cognitive decline. Strike et al. (2015) found a positive result in processing speed and verbal recognition memory using a commercial product from the company Efamol, which combined DHA + EPA + *Ginkgo biloba* + phosphatidylserine + vitamin E + folic acid + vitamin B12. This result supports the idea that a combination supplement may be superior, although

if the authors had also included a DHA + EPA capsule only this may have provided some insight into the issue.

Nine trials used a higher daily amount of DHA than EPA, five trials used a higher daily amount of EPA than DHA, two used the same or similar amount of DHA and EPA, four used DHA only, one used DHA and ARA with no EPA, one used a ratio of DHA:EPA of 3:1, and one included two fish oil groups, one in which the EPA content was higher and one in which the DHA was higher. Yacong et al. (2017) suggested that the ratio of EPA to DHA of 3:2 is an important factor, and used a dose of 720 mg of EPA and 480 mg of DHA per day for 6-months. Whether this ratio and dose were responsible for why Yacong et al. found a significant difference in three out of five subtests is unclear. One trial used a 5-week cross-over design (Nilsson et al., 2012) which may not be the most appropriate design for a fish oil trial when considering that *n*-3 PUFAs can be stored in the body for up to 2.5 years (Stonehouse et al., 2013). In contrast, the trial by Quinn et al. (2010) used an algal supplement, containing a daily dose 1000 mg of DHA with no EPA (or any other ingredients), in older adults with AD with low baseline levels of DHA. In this medium sized trial (402 participants started, 295 completed), DHA supplementation did not slow the rate cognitive or functional decline, except for a subgroup of non-ε4 carriers.

van de Rest et al. (2008) and Dangour et al. (2010) both chose daily doses of DHA and EPA to reflect national dietary recommendations and what is 'achievable' through diet as opposed to a pharmacologic dose. Pharmacologic refers to a dose that is larger or more potent than what would occur naturally so as to have a different effect. In fact, van de Rest et al. included both a low (to reflect dietary recommendations) and high (pharmacologic) dose group and found no difference between either *n*-3 PUFA dose group and the placebo

group, which is consistent with the finding by Mazereeuw et al. (2012) that dose was not related to treatment effect.

APOE genotyping was included in five of the trials – Freund-Levi et al., 2008; van de Rest et al., 2008; Quinn et al., 2010; Külzow et al., 2016; and Danthiir et al., 2018. Freund-Levi et al. found ε4 carriers showed a significant treatment effect in agitation, van de Rest et al. found a treatment effect in the attention domain for ε4 carriers and Quinn et al. found lower decline in the ADAS-cog for non-ε4 carriers. Külzow et al. found recall performance was significantly better in the fish oil group after controlling for dietary habits and APOE ε4 carrier status, and Danthiir et al. 2018 found a greater improvement from fish oil in the domain of reasoning for ε4 carriers. Baseline RBC or plasma DHA and EPA levels were tested in the majority of the 23 trials, with the omega-3 index and 50^{th} percentile used to determine low and high baseline levels of DHA. Most trials used this data to determine the change in DHA and EPA levels from baseline to end, as opposed to using baseline DHA levels as a covariate in the final analysis.

Several researchers have suggested that the different results between trials may be due to differences in DHA and EPA levels of participants at baseline. The results from the trial by Quinn et al. do not support this idea, although the authors quantified a 'low' baseline level using a 7-item FFQ as opposed to baseline RBC or plasma DHA levels. Confounding variables such as a healthy lifestyle and life circumstances, including socio-economic and educational level, are associated with higher fish consumption and lower dementia risk, and may explain the positive findings in some trials. The evidence from epidemiological studies on the beneficial effects of fish consumption provides supports for this. Again, this highlights the importance of assessing the baseline levels of DHA in fish oil trials as well the need for a standard blood sampling procedure. The lack of standardisation

across studies from the cognitive measures to the sampling tissue (plasma vs. RBC) through to the reporting of *p* values as opposed to effect sizes contributes to a very confusing picture for those trying to decide whether taking a fish oil supplement is worthwhile or not.

While the trials by Lee et al. (2013), Rondanelli et al. (2012) and Yacong et al. (2017) were not included in the meta-analysis by Mazereeuw et al. (2012), they provide further support for the beneficial effect of *n*-3 PUFAs in older adults with MCI. It appears that somewhere between cognitively healthy and dementia is where fish oil supplementation may be most beneficial, although individual differences both genetically and in terms of previous intake of *n*-3 PUFAs, may also be important factors. Except for the two German trials (Witte et al., 2013; Külzow et al., 2016), the source of *n*-3 PUFAs, the dose and ratio of DHA and EPA, the inclusion of bio-factors and the duration of the trial varies widely between the trials. Whether or not any of these factors can explain the conflicting results is unclear.

CHAPTER 6

DESIGNING THE TRIAL

Based on the results of the meta-analysis by Mazereeuw et al. (2012), the decision was made to investigate the cognitive effects of a fish oil supplement in older adults with Mild Cognitive Impairment (MCI) by replicating components of previous trials and with the inclusion of APOE genotyping. At the time of designing the trial, two fish oil trials were known to have specifically investigated the effects of a fish oil supplement in older adults with MCI – Sinn et al. (2012) and Lee et al. (2013). The decision was made to replicate these two trials by using a similar dose of DHA, with the inclusion of several of the same measures, and in the case of the trial by Lee et al., with the same trial duration. The decision was also made to extend these two trials by adding a 9-month testing time point and by including APOE genotyping.

As part of designing the current RCT, many decisions had to be made regarding the type of fish oil supplement, the choice of placebo, the number of testing time points or sessions, what to test and how to measure it, as well as how to analyse and interpret the results. This chapter provides explanations for each of these design decisions as well as discussing other important parts of the research process including applying for funding, and correspondence with the supplement company and laboratories as part of the project management and administration duties.

6.1 Duration of the trial

The trial by Sinn et al. (2012) was based on a 6-month supplement period and found a significant difference between the DHA and placebo groups in one of the cognitive tasks, Initial Letter Fluency, and on the GDS at the 6-month mark. The trial by Lee et al. (2013) was based on a 12-month supplement period and found a significant difference between the fish oil and placebo groups in three of the cognitive tasks, Digit Span Backwards, Visual Reproduction I and Rey Auditory Verbal learning test, at the 12-month mark. Based on these results, it seemed that 6-months of supplementation may be a 'minimum supplement period', and therefore anything less would not be sufficient to see any significant changes in the cognitive outcomes. The decision was made to use a 12-month supplement period with a 6-month testing mark as well as a further testing point at the 9-month mark. This 9-month testing time point would be used to investigate any cognitive changes during the six month period between 6-months and 12-months of DHA supplementation. In particular, to explore the idea that there may be a plateau effect around the 9-month mark, with an improvement from baseline to 9-months, but with no further improvements from 9-months to 12-months.

6.2 Capsules

The DHA group in the Sinn et al. (2012) trial were given a daily dose of 1550 mg of DHA plus 400 mg of EPA for six months, and the fish oil group in the Lee et al. (2013) trial were given 1300 mg of DHA plus 450 mg of EPA per day for 12 months. The decision was made to use a fish oil supplement that contained a similar amount of DHA and EPA as these two trials. A major consideration when choosing a fish oil supplement for the trial was that participants were likely to be taking other tablets or capsules during the trial period. Therefore, the fish oil supplement should ideally deliver a high daily dose of DHA in a

minimum number of capsules, and in a small capsule size. The decision was made to use an oblong shaped, size 20, 15 mm length, soft-gel capsule which is about the size of a standard oblong paracetamol tablet. This size of capsule can hold up to 1000 mg of oil per capsule, and so for a dose of three capsules per day, each capsule would need to contain at least 400 mg of DHA. The advantage of a soft-gel capsule is that the rounded ends of the capsule can reduce swallowing difficulties. The only issue when using a soft-gel capsule is that if swallowed with a hot liquid they can start to dissolve while still in the mouth. To reduce the likelihood of this happening, participants would be asked to use room temperature or cold liquids when swallowing the capsules. Participants would also be asked to take the capsules after breakfast, as taking capsules after food reduces the chances of reflux. The capsule would also need to contain citrus oil to mask the flavour of the fish oil and to act as an emulsifier to assist with the digestion of fatty acids, and an anti-oxidant agent such as vitamin E.

To further replicate the two trials by Sinn et al. (2012) and Lee et al. (2013), the decision was made to use a placebo that contained similar amounts of the *n*-6 PUFA Linoleic acid (LA). The placebo group in the trial by Sinn et al. were given a daily dose of 2200 mg of LA for six months by way of safflower oil, and the placebo group in Lee et al. were given 1800 mg of LA per day for 12 months by way of corn oil. Despite the use of safflower and corn oil in these two trials, most other fish oil trials have used either olive oil or sunflower oil as the placebo oil. Olive oil is high in *n*-9 PUFAs, with very little *n*-3 PUFAs and *n*-6 PUFAs, and is often used to reduce and therefore control for the effects of the placebo on the ratio of *n*-3:*n*-6 (Danthiir, Burns, Nettelbeck, Wilson, & Wittert, 2011). In an attempt to replicate the two trials by Sinn et al. and Lee et al., sunflower oil was chosen as the placebo oil as a

1000 mg capsule would be equivalent to approximately 600 mg of LA (with no trace of DHA or EPA), and therefore three capsules per day would deliver approximately 1800 mg of LA.

After researching the types of fish oil products that were available on the market and then contacting fish oil supplement manufacturers and suppliers within NZ to find a fish oil supplement with the above specifications, the possibility of asking a fish oil company to supply the capsules for the trial to reduce costs was explored. However, after further research, a supplement that would provide enough DHA and EPA in less than five capsules per day could not be found or would take over a year to arrange. Because a year was too long to wait, the decision was made to pay for the capsules. A pharmaceutical company based in Auckland NZ, GMP pharmaceuticals, eventually confirmed that they were able to provide a fish oil supplement with a DHA:EPA ratio of 5:1, equivalent to 500 mg of DHA and 100 mg of EPA in a size 20, soft-gel capsule, meaning that three capsules per day would deliver approximately 1500 mg of DHA and 300 mg of EPA. GMP pharmaceuticals also confirmed they were able to provide a sunflower placebo capsule, identical in size, shape and composition except for the active oil. See Table 9 for the initial capsule composition specifications.

Based on these specifications, this dose of DHA and EPA would be considered ‘pharmacologic’, in that it is larger or more potent than what would be deemed ‘natural’ or ‘normal’. This dose would be equivalent to eating approximately four to five portions of oily fish per week, and is above what would be considered ‘achievable’ through diet.

Table 9. Capsule composition (mg/per capsule)

Ingredients		**Per capsule**
Actives	Oil	1000
Excipients	Vitamin E 1300 IU/g	1.15
	Orange oil	10
	Fill weight	1011.15
Shell	Gelatin	240
	Glycerol	120
	Caramel	2
	Water – purified	38
	Shell weight	400
	Total weight	1411.15

6.3 Medico-paks vs. opaque bottles

As there was concern about capsule compliance during the long trial period, the use of Medico-paks was considered as a way of dispensing the capsules. Medico-paks are often used with older adults to help keep a record of whether capsules or tablets have been taken on a particular day or not. Douglas Pharmaceuticals Ltd, a supplier of the packs, was contacted regarding the possibility of using the packs for the trial. Medico-paks are typically packed by a pharmacist, however Douglas Pharmaceuticals could supply the packaging to be packed on site. Medico-paks are not airtight, and therefore, for a supplement like fish oil which is sensitive to heat and light, they could only be inside a Medico-pak for maximum of one month. This meant that the capsules would need to be packaged every month, which would be both costly and difficult to orchestrate. Instead, it was decided to dispense the capsules in sealed, white, opaque bottles as they could be bottled by the manufacturer without labels but with an expiry date and batch number printed on the bottom. They would be labelled by a research assistant, and then easily stored in the refrigerator by the participants. The fish oil capsule batch number was 294 and the placebo batch number was

295. These batches numbers were removed by a research assistant once they had been labelled.

6.4 APOE genotyping and RBC fatty acid profiles

The decision to include APOE genotyping and RBC fatty acid profiles was in line with the decision to extend and replicate the two fish oil trials. The decision was made to test APOE status at the end of the trial, so the lead researcher could remain blinded to APOE status until the end of testing and to minimise the cost from participant drop-outs due to high cost of APOE genotyping ($157 per sample). The main concern of the Health and Disability Ethics Committee (HDEC) was how the results of APOE genotyping results would be explained to participants. An expert in APOE genotyping advised that the key piece of information to convey to participants was that APOE genotyping is not a diagnostic genetic test; if a participant is found to carry a ε4 allele, it does not mean that this person will develop AD, rather it indicates an increased risk of developing AD. Further, it would be important to explain to ε4 carriers that many other factors contribute to the development of AD, while there are also other factors that offer protection from the disease. The HDEC were satisfied with this procedure as well as with the APOE factsheet that would be sent to ε4 carriers. The HDEC fully approved the trial on the 28th of October 2014.

6.5 Participants

A power analysis was performed using G*Power 3.1.9.2 (Faul, Erdfelder, Lang, & Buchner, 2007) to calculate the required number of participants. Based on the calculation of 4 groups (Fish oil/APOE ε4, Fish oil/not APOE ε4, Placebo/APOE ε4 and Placebo/not APOE ε4) using an ANCOVA (with fixed effects, main effects and interactions) with an estimated

effect size of f = 0.40, to provide a statistical power equal to 0.8 and a α level of 0.05 (2-tailed), the minimum total sample size required was 64, 16 participants in each of the four groups.

The decision was made to focus the trial on participants with Mild Cognitive Impairment with an age range of 65 to 95 years of age, based on Statistics NZ's (New Zealand's official data agency) definition of older people as those aged 65+. This age range would allow comparison of statistical data, and because this is the age of retirement in NZ, would mean participants would be more likely to have time during the day to attend the testing sessions. However, due to a slowdown in recruitment, the decision was made to alter the age range from 65 to 60 years of age and from 95 to 90 years of age. The main issue with an age range of 60 to 95 years is that it would have spanned four decades, therefore the final decision was to reduce this to three decades and to use age as a covariate in the final analysis.

Recruitment for the trial began in September 2015. Based on the inclusion and exclusion criteria, a screening questionnaire (see Appendix D) was developed to be administered via a 10-minute phone call. By January 2016, after four months of recruitment and screening, only 35 participants (the majority had been recruited from a previous memory study) had been enrolled so the decision was made to include 60 to 64 year olds. To increase participant numbers, advertisements, posters and leaflets were placed around Wellington. All advertisements and announcements specified that the study was looking for people who were interested in being involved in a PhD research project that looked at ageing and memory, who were over the age of 60 and who thought they might have a mild memory problem. See Appendix E for the trial leaflet and advertisement. See Appendix F for the participant information sheet and consent form. Two clinical psychologists from the

Older Persons and Rehabilitation Service of Hutt Valley Hospital, and the Neurology department at Capital Coast District Health Board also agreed to help recruit participants. As part of an email update sent through to participants in February 2016, participants were asked if they knew anyone who might be interested in taking part in the trial.

6.6 Location

Because participants were to be tested at four time points, the decision was made to base the trial at the Massey Psychology Clinic in Mount Cook, Wellington. This ensured that participants would not be required to travel more than one hour (one way) to the clinic. Participants were given a map specifying the location of the clinic and the Wellington hospital outpatients unit (for blood sample collection) along with the information sheet and consent form. Two participants who expressed reservations about participating in the trial, one due to a recent injury and the other due to the time required for them to travel to the clinic, were tested at their homes. Another participant was tested in their home for the final testing session due to deterioration in their physical health throughout the course of the trial.

6.7 Measures

The meta-analysis performed by Mazereeuw et al. (2012) found that the biggest improvement in cognitive performance from fish oil supplementation was in delayed verbal recall, immediate recall, attention and processing speed. As a result, the cognitive battery needed a heavy emphasis on measuring memory (allowing differentiation between memory tasks that depend on attention and processing speed and those which do not), along with other main cognitive domains including language, attention, executive functioning, motor

function, and because of the participant characteristics, pre-morbid functioning. Research indicates that discriminating between MCI and cognitively healthy older adults requires both a word list recall test and an executive functioning task. The cognitive battery would also need to be brief and easy to administer. Sinn et al. (2012) used a cut-off point of 1.5 SDs for measuring the cognitive impairment, whereas Lee et al. (2013) used a cut-off point of 1 SD. Thus, the decision was made to use between 1 and 1.5 SDs in at least one cognitive domain.

Four cognitive measures were considered – the Rivermead Behavioural Memory Test (Wilson, Cockburn, & Baddeley, 1991), Addenbrooke's Cognitive Examination-Revised (ACE-R; Mathuranath et al., 2000), the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005) and the RBANS (Randolph, 1998)**.** The RBANS was chosen because of the availability of age norms, particularly for the 80 to 89 year age group, and because it had four alternate forms – RBANS A, B, C and D, which meant a different version could be used at each testing session. In addition, the RBANS is easy to administer, and each form of the RBANS involves two immediate memory and four delayed memory tasks resulting in both an immediate and delayed index score. Several of the participants were recruited from a previous memory study where the RAVLT had been used as the primary measure of memory, and therefore the RBANS provided an alternative version of a word list recall test to reduce any practice effects.

The tasks in the RBANS are organised into five indexes – Immediate Memory, Visuospatial/Constructional, Language, Attention and Delayed Memory. According to the RBANS manual, reliability coefficients for the five indexes for the age groups 60 to 69, 70 to 79 and 80 to 89 years of age respectively are: .85, .89 and .90 for Immediate Memory, .84, .81 and .78 for Visuospatial/Constructional, .85, .81 and .83 for Language, .83, .88 and .85 for Attention and .85, .83 and .81 for Delayed Memory. Randolph (2012) commented that

reliability coefficients of the RBANS indexes are higher in older adults than in young age groups, perhaps due to the ceiling effects and reduced variability in younger controls. Also noted is that the reliability coefficients tend to be higher for the indexes than for the subtests. For example, the subtest coefficients for the age group 80 to 89 are: .84 for List learning, .84 for Story Memory, .54 for Figure Copy, .52 for Semantic Fluency, .83 for Digit span, .83 for Coding, .72 for Story Recall and .55 for Figure Recall. Although a closer inspection shows that this is probably only an issue for Figure Copy and Recall, and the Semantic Fluency tasks. In terms of validity, the total RBANS score correlates well with the Wechsler Memory Scale (WMS-R) Verbal Memory Index (*r* = .70), the Wechsler Adult Intelligence Scale (WAIS-R) FSIQ short form (*r* = .78) and the WMS-R Attention/ concentration index (*r* = .84) (Randolph, Mohr & Chase, 1993). The WMS-R Attention/concentration index also correlated well with the RBANS Attention index (*r* = .82) (Randolph, 2012). In terms of measuring executive functioning, the decision was made to include both the Trail Making Test and a version of the Stroop Test. The big issue with the RBANS as a cognitive battery is that adjustments for gender and education are not available, yet research has shown that both result in modest to large effects on RBANS scores (Beatty, Mold, & Gontkovsky, 2003).

Measures of depression, anxiety and health-related quality of life were also included to allow comparison with other fish oil trials. The Geriatric Depression Scale (GDS; Sheikh, & Yesavage, 1986), the Geriatric Anxiety Inventory (GAI; Pachana, Byrne, Siddle, Koloski, Harley, & Arnold, 2007) and the 36-Item Short Form Health Survey Questionnaire (SF-36; Ware & Gandek, 1994) were selected because Sinn et al. (2012) used both the GDS and SF-36, because the GDS and GAI are specifically developed to measure depression and anxiety in older adults, and they are both brief to administer and easy to score. The decision was

made to only use a brief *n*-3 PUFA nutritional screening questionnaire at baseline because the RBC fatty acid profiles would provide a measure of changes in dietary fatty acids including *n*-3 PUFAs and *n*-6 PUFAs for the 12-month supplement period. Cardiovascular biomarkers by way of height and weight in order to calculate BMI, as well as systolic and diastolic blood pressure were included because of the link between cardiovascular disease and cognitive decline such as vascular dementia. The link between cognitive decline and weight is also of interest, as weight loss is considered a clinical expression of cognitive decline and dementia, and to worsen the clinical course of dementia.

A measure commonly used in clinical trials to improve compliance is a daily diary or charting system. Because participants in the trial were older adults, a traditional calendar style format was considered to be the most appropriate. This was confirmed after a small pilot study with a template revealed that it was easy to understand and use, and was something that people would consider putting up in the kitchen or somewhere likely to be seen every day. The calendar had a front page containing the short title of the study, the Massey University logo and a space for the participant's name and ID number. The second page explained the purpose of the calendar along with details of how to use it. The following pages included one page for each month of the 12-month supplement period. As well as assisting with and assessing capsule compliance, the calendar would keep a record of fish consumption and any illnesses throughout the trial. See Appendix G for an example page. A standard operating procedures document was also developed, outlining the test order along with descriptions and instructions for each measure to ensure consistency and to standardise the order of testing for a research assistant to follow.

6.8 Project management and administration

In this section, the project management and administration components of the trial will be described, including applications for funding, training of research assistants as well as correspondence with the participants. The section will begin with two aspects of the project management and administration, both of which were very challenging at times – correspondence with GMP pharmaceuticals and correspondence with Wellington Regional hospital laboratory. See Figure 3 for a chart of the project management time frame.

6.8.1. GMP pharmaceuticals and the capsules

Communications with GMP pharmaceuticals began in July 2014 with monthly emails about capsule specifications and costing. Once the capsule specifications were decided on, it was calculated that each participant would need a total of 1,095 capsules for the 12-month supplement period. In addition, to ensure that participants never ran out of capsules and to accommodate any dropped or lost capsules, it was decided that each participant would be dispensed 1,200 capsules divided into 6 bottles, each containing 200 capsules. Based on the calculation that each participant would need 1,200 capsules, a total of 96,000 capsules would be needed for a sample size of 80. GMP Pharmaceuticals advised that capsules could be ordered in batches of 100,000 capsules, but because of the types of oils that had been chosen, the minimum order of 200,000 capsules would be required.

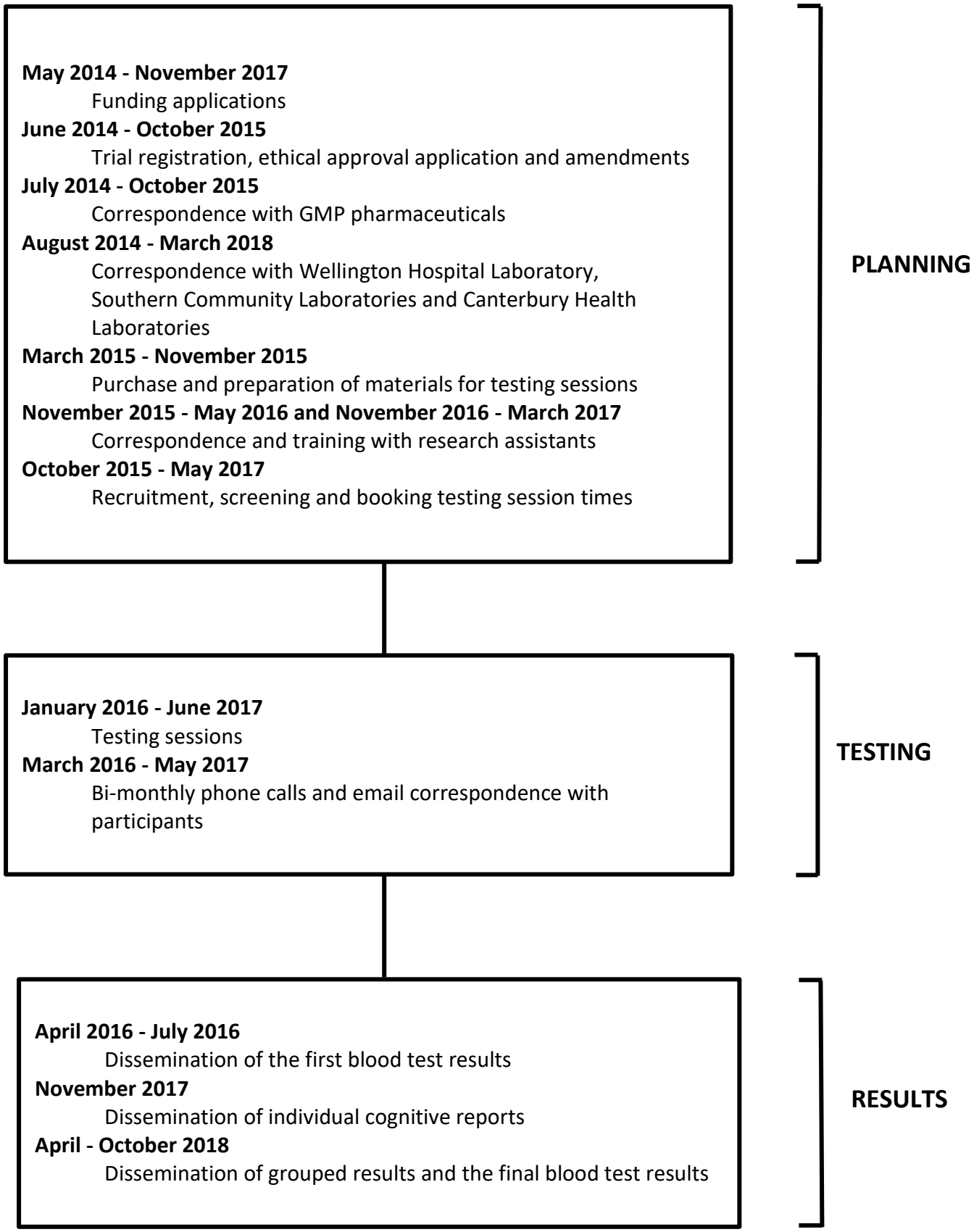

Figure 3. Chart outlining the time frame of project management and administration tasks

The total cost for 200,000 capsules was set at $16,985.50. Because of the major delays already experienced in funding and recruitment, the decision was made to place the order, knowing that more funding would be needed to cover the expense. Further delays from GMP meant the trial start date was further delayed, and consequently materials such as the calendars needed to be reprinted. GMP finally confirmed a delivery date and so the trial start date was set.

The capsules were independently tested in order to report the exact fatty acid composition. A sample of the two types of capsules was sent to the Nutrition laboratory at the Institute of Food, Nutrition and Human Health, Massey University in Palmerston North for fatty acid profile analysis. A report indicated that the levels of DHA and EPA were 10% below what had been initially agreed upon with GMP. Legal advice from two legal representatives at Massey University advised to seek compensation for the discrepancy. GMP replied to the legal action with test results, which contradicted those of the Nutrition laboratory. The capsules were then re-tested by Cawthron Analytical Services, at the Cawthron Institute in Nelson. This time the results showed that the DHA and EPA levels were similar to both what GMP had originally specified, and to the test results GMP had provided. After correspondence with several laboratory technicians, it was probable that the discrepancy was a result of different analysis procedures.

6.8.2 Correspondence with laboratories

Communications with Wellington Regional Hospital began in August 2014, resulting in a meeting with the team leader of client services at the hospital laboratory in October 2014. It was arranged that the laboratory would organise to collect, analyse and transport the blood samples for the trial, and provided a quote outlining the costs of these services.

One of the major obstacles during this time was that the analysis needed for RBC fatty acid profiles was not a routine laboratory test. As a result technicians at the Wellington hospital laboratory and the Nutrition Laboratory at the Institute of Food, Nutrition and Human Health at Massey University had to work out what the analysis would involve, and which parts of the procedure each laboratory would do. A patient form was developed to include the details of the lead researcher (in case a Phlebotomist had any queries), the details of the trial, instructions for the Phlebotomist regarding the tubes required and the analysis to be performed. See Appendix H for the patient form.

A major overhaul of the Wellington hospital laboratory and the incorporation of Southern Community Laboratories (SCL) took place the following year which caused two major changes. The first was an increase in cost, the second was access to collection centres other than at the outpatients unit of the Wellington Hospital. This meant that participants were able to have their blood samples taken at one of the SCL collection centres in and around Wellington. However, as a result of this restructuring, several samples went missing, or were not taken or stored properly, or in five cases destroyed due to an explosion in the main hospital laboratory. Several participants were asked to give repeat samples, and one participant had to have blood taken a total of five times (instead of twice) due to these issues.

6.8.3 Funding, assistants and participant phone calls

The two biggest expenses of the trial were the capsules and blood test collection and analysis. During the four years, over 30 scholarship and funding applications were submitted. Several were successful and the trial received $20,000 from the Oakley Mental Health Foundation in March 2015, $11,647 from the Neurological Foundation in July 2015,

$18,000 from the Hope-Selwyn Scholarship of Ageing Research and $9,490 from a Massey University Research Fund in January 2018.

Two post-graduate psychology students assisted with two different components of the trial. The first research assistant helped with the randomisation procedure and labelling of the capsule bottles, and the second assistant helped with testing participants during the 3rd testing session. For the randomisation procedure, a procedural document was developed explaining step-by-step the process of generating a list of random participant numbers, assigning the participant numbers to one of the two capsule groups, labelling the capsule bottles and then removing the batch numbers from the bottom of the bottles. The first assistant was the only person to know which group the participants had been allocated to – the lead researcher, supervisors and second assistant were not aware of the participant group allocations until after the final testing session. The first assistant was reimbursed with eight $20 supermarket vouchers.

The second research assistant had completed a post-graduate course in clinical neuro-psychology and had some familiarity with psychometric tests. The assistant was first briefed, and then trained on administering and scoring the cognitive and well-being tasks. This involved two initial meetings, firstly outlining the trial design and the individual tests, and providing a copy of the standard operating procedures to read over, and secondly, a follow-up meeting where the testing schedule and availability were discussed. The research assistant then observed while the lead researcher administered the tests to a clinical psychology student and then administered the tests to the same clinical psychology student themselves. In total, the second assistant tested 45 of the final 60 participants, all with version C of RBANS at the third testing time point and was reimbursed with $500 in cash.

As well as the screening phone calls and testing sessions, the main form of communication with participants was via bi-monthly phone calls. During the first two of these phone calls, the aim was to ensure participants were not experiencing any serious side effects or problems with swallowing the capsules. If the side effect was deemed as too severe to continue, the participant was immediately withdrawn. If the side effect was less severe (e.g., minor reflux), suggestions were made in terms of changes in how and when the capsules were taken, which in most cases reduced the side effect. The blood test results were also given to participants via these bi-monthly phone calls. Later phone calls included updates on how the trial was progressing, when future testing sessions would take place, booking testing sessions as well as asking if the participant had noticed any physical or cognitive changes as a result of taking the capsules.

6.9 Aims and hypotheses of the trial

To conclude this chapter, the aims and hypotheses of the trial will be outlined again. The primary aim of the trial was to investigate the effects of a 12-month DHA fish oil supplement on cognitive performance in older adults with MCI. It was hypothesised that after 12 months of supplementation, participants in the DHA group would show higher scores in the immediate recall, delayed recall and working memory tasks than participants in the placebo group. The secondary aim of this trial was to investigate whether the APOE ε4 allele has a moderating effect on the measures, as well as to examine the effect of the fish oil supplement on well-being, in particular depression. It was hypothesised that carriers of the APOE ε4 allele would benefit from (taking) the DHA supplement, and that the DHA group would show an improvement in depression scores.

CHAPTER 7

METHOD

7.1 Participants

A total of 183 people were screened for the trial and 87 older adults were found to fit the eligibility criteria. Of the 87 older adults, 15 were excluded at baseline, which resulted in a final sample size of 72 participants.

7.1.1 Recruitment

Recruitment for the trial began in September 2015, and was completed at the end of May 2016. The first group of older adults was recruited from another study through the School of Psychology at Massey University in Wellington, which investigated memory complaints in adults over the age of 40 living in Wellington, NZ. Participants from this study were asked if they would be interested in participating in another research study, and if so their name and contact details were passed on to the lead researcher. The second group of older adults was recruited through advertisements in local newspapers, posters placed in libraries, hospitals and supermarkets around Wellington, leaflets and announcements through the Wellington City University of the Third Age (U3A), Probus, Grey Power, SeniorNet, Age Concern and the website 'GrownUps', as well as through Wellington District Health Boards, and word-of-mouth invitations. Of the 183 people screened for the trial – 64

people came from the previous Massey University memory study, 40 from posters and leaflets around Wellington, 37 from word-of-mouth, 18 from newspaper advertisements, 14 from U3A, eight from Probus, Grey Power, SeniorNet, Age Concern and GrownUps, and two from the Older Persons and Rehabilitation Service of Hutt Hospital.

7.1.2 Screening

Screening was conducted via a 10 to 15 minute phone call. During this phone call potential participants were given a brief description of the trial which included an explanation of the time-frame as well as the randomisation and double-blind elements of the study, and then asked if they would be willing to take three capsules per day for a 12-month period and attend four testing sessions at the psychology clinic at Massey University over the course of a 15-month period. If participants answered 'yes', they were asked if they were between 60 and 90 years of age, if they had regularly taken any fish oil supplements over the previous 6-month period, if they consumed more than two servings of fish per week and if they were willing not to start taking fish oil supplements of their own during the study.

Participants were then asked whether they would be away from Wellington for any period of time during the next 15 months, if they were willing to have two blood samples taken throughout the trial and whether they lived independently in their own homes. Participants were asked whether they had any major medical conditions including head injury or any recent or upcoming major surgery, about current medications in particular whether they were taking the blood-thinner medication warfarin, whether there were any problems with swallowing soft-gel capsules or an allergy to any type of fish or seafood. Finally, potential participants were asked whether they or their family or friends had noticed

in change in their memory or cognition in the last six to 12 months. If participants answered ‘no’, they were excluded and were told the reason why, if answered ‘yes’, they were asked to explain further.

Participants were sent the information sheet and consent form in the post, and then phoned approximately two weeks later to discuss and answer any questions about the trial. Participants then completed the consent form and returned it using a postage-paid envelope. From the 183 people screened, 96 were excluded – 34 people regularly took fish oil supplements, 11 reported no subjective memory or cognition complaints, eight were on contraindicated medications, seven ate more than two servings of fish per week, five were vegetarian or vegan, five had travel plans during testing times, five felt their health was too poor to participate, four wanted to start taking a fish oil supplement of their own, four did not want to swallow capsules, three had heavy work commitments, three felt the trial was too long to commit to, two had sustained head injuries, two were older than 90 or younger than 60 years of age, two lived outside of Wellington and one person did not want to be involved in the trial for other reasons. See Figure 4 for a flowchart outlining the process of participant recruitment and screening for the trial.

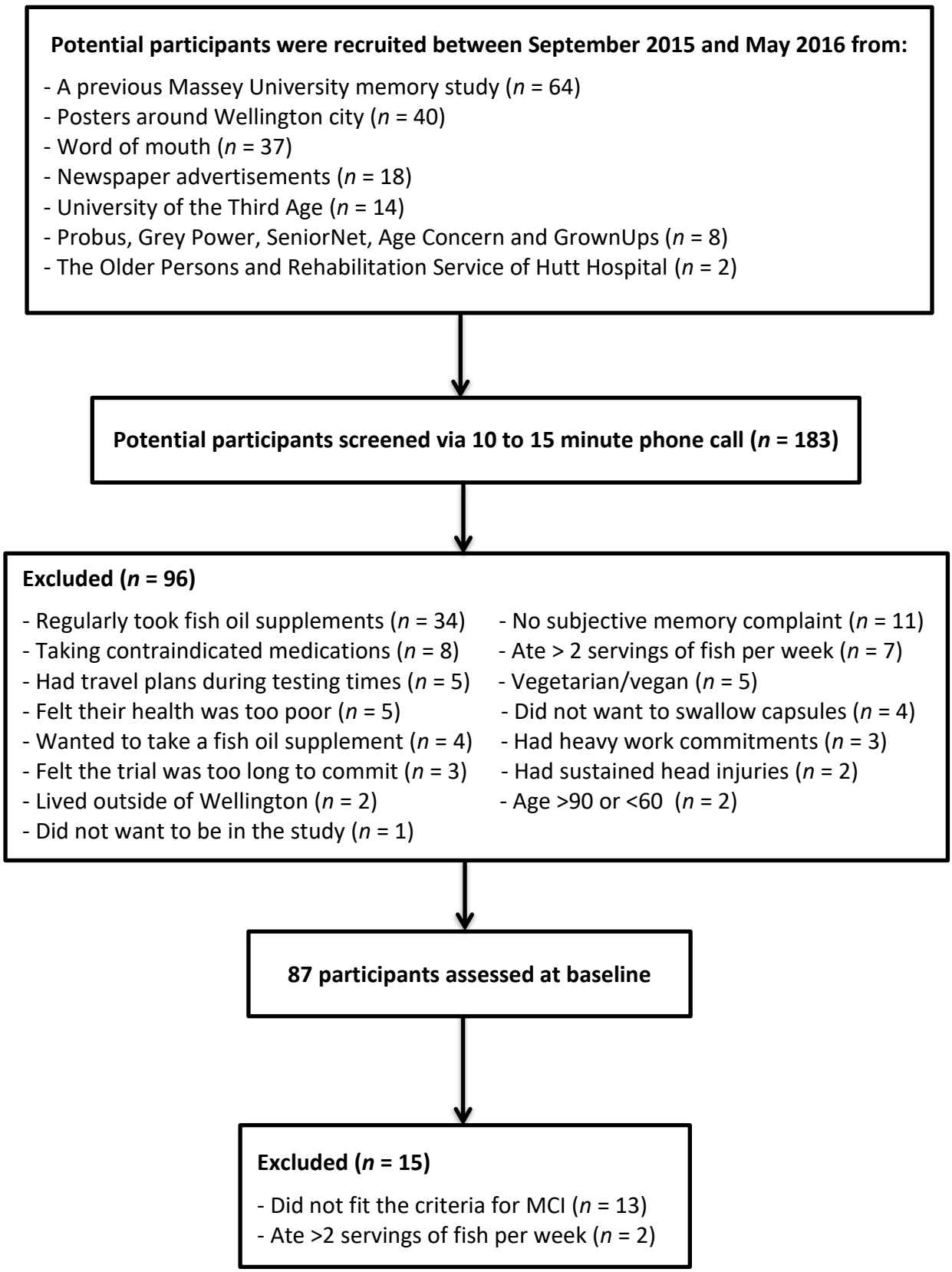

Figure 4. Participant recruitment and screening

7.2 Classification of Mild Cognitive Impairment

Participants were classified as having Mild Cognitive Impairment (MCI) based on the following criteria by Petersen (2004): 1. Presence of a memory complaint. 2. Evidence of cognitive impairment in at least one cognitive domain as assessed as a score or scores between 1 to 1.5 standard deviations below the mean for age. 3. Preserved general cognition i.e., normal IQ. 4. Ability to perform everyday activities without the assistance of another person. 5. Not demented.

At the baseline testing session a total of 87 older adults were tested. Fifteen were excluded – 13 because they did not fit the criteria for MCI based on criteria 2, and two were excluded because after completing the nutritional questionnaire it was found that their seafood intake was more than two servings per week.

7.3 Trial registration and ethical approval

The trial was registered with the Australian New Zealand Clinical Trials Registry ACNTRN12614000874617 on the 14th of August 2014 with updates made on the 2nd of November 2015 and 6th of October 2016. Ethical approval for the trial was obtained by the Central Health and Disability Ethics Committee on the 28th of October 2014. Ethics Reference: 14/CEN/148. Minor amendments were made to the trial design after peer review and these changes were approved by the Central Health and Disability Ethics Committee on the 30th of April 2015 and 30th of October 2015. Ethics References: 14/CEN/148/AMO1 and 14/CEN/148/AMO2. See Appendix I. Annual ethics progress reports were approved on the 18th of February 2016 and 28th of September 2017.

7.4 Study design

A 12-month parallel, randomised, double-blind, placebo-controlled, interventional study design was used. The participants were divided into two groups to assist with administration and testing. For the first group the 12-month supplement period began on the 1st of March 2016 and finished on the 28th of February 2017, and for the second group the 12-month supplement period began on the 1st of June 2016 and finished on the 31st of May 2017. The cognitive tasks and well-being measures were assessed at four time points – at baseline, then after 6-months, 9-months and 12-months of taking the capsules. These testing sessions were also referred to as the baseline, 2nd, 3rd and final testing sessions.

7.5 Intervention

The DHA group were given three capsules per day, each capsule contained 1000 mg of fish oil equivalent to 497 mg of DHA, 117 mg of EPA, 1.15 mg of Vitamin E 1300 IU/g and 10 mg of orange oil, equating to a total daily dosage of 1491 mg of DHA and 351 mg of EPA. The placebo group were also given 3 capsules per day, each capsule contained 1000 mg of sunflower oil equivalent to 619 mg of LA, 1.15 mg of Vitamin E 1300 IU/g and 10 mg of orange oil, equating to a total daily dose of 1857 mg of LA per day. Both types of capsules contained vitamin E as an anti-oxidant, and orange oil to help mask the taste of the fish and sunflower oils. Table 10 shows the fatty acid composition of each capsule type by way of grams per 3000 mg daily dosage. Both types of capsules were supplied by GMP Pharmaceuticals Ltd and were independently tested by Cawthron Analytical Services, at the Cawthron Institute in Nelson. The dose of DHA and EPA was based on the two trials by Sinn et al. (2012) and Lee et al. (2013) and was equivalent to eating approximately 4-5 portions of oily fish per week.

Table 10. Fatty acid composition of DHA and placebo capsules (g/3000 mg daily dosage)

Fatty acids	DHA capsules	Placebo capsules
Palmitic acid (C16:0)	0.022	0.174
Stearic acid (C18:0)	0.022	0.088
Oleic acid (C18:1*n*-9)	0.045	0.866
Linoleic acid (C18:2*n*-6)	<0.01	1.857
Alpha-linolenic acid (C18:3*n*-3)	<0.01	<0.01
Eicosenoic acid (C20:1*n*-9)	0.047	<0.01
Arachidonic acid (C20:4*n*-6)	0.036	<0.01
Eicosapentaenoic acid (C20:5*n*-3)	0.351	<0.01
Docosapentaenoic acid (C22:5*n*-3)	0.107	<0.01
Nervonic acid (C24:1*n*-9)	0.057	<0.01
Docosahexaenoic acid (C22:6*n*-3)	1.491	<0.01

The two types of capsules were identical in size, colour and shape. Participants were asked to take the three capsules every day after breakfast with water for the course of the 12-month supplement period. The capsules were dispensed in white opaque plastic bottles, with each bottle containing 200 capsules. Each participant received six bottles in total. Each bottle was labelled with the participant's name, participant number, the required dose and directions, storage instructions, the batch number and expiry date. The first four bottles were given to the participant at the end of the baseline testing session, the fifth bottle at the end of the 2nd testing session and the sixth bottle at the end of the 3rd testing session. Participants were asked to store the bottles in the refrigerator, and to bring any unopened or partially full bottles to the following testing session for counting as part of the compliance assessment.

Participants were randomly allocated to one of the two capsule groups using a randomly generated list of numbers from Random.org. A paid research assistant generated the list of random participant numbers, assigned the participant numbers to one of the two capsule groups, and labelled the capsule bottles.

7.6 Cognitive measures

The battery of cognitive tasks used to assess cognitive performance were the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998)**,** the National Adult Reading Test (NART; Nelson, 1982), the Coin Rotation Task (CRT; Mendoza, Apostolos, Humphreys, Hanna-Pladdy, & O'bryant, 2009), the California Older Adult Stroop Test (COAST; Pachana, Thompson, Marcopulos, & Yoash-Gantz, 2004), the Trail Making Test A and B (TMT; Army Individual Test Battery, 1944) and the Digit Span Backwards Test (DSBT) from the Wechsler Adult Intelligence Scale 3 (WAIS-III; Wechsler, 1997). This battery of tasks focused on assessing all cognitive domains including attention, memory, language, visuo-spatial, executive functioning, information processing speed, motor function and pre-morbid ability, with an emphasis on the main types of memory, especially those that are affected by early age-related cognitive decline such as episodic memory.

7.6.1 Repeatable Battery for the Assessment of Neuropsychological Status – RBANS

The RBANS is a neurocognitive battery with four alternate forms – RBANS A, B, C and D. Form A was used at the baseline testing session, form B at the 2nd testing session, form C at the 3rd testing session and form D at the final testing session. Each alternate form consists of 12 subtests divided into five indexes – Immediate Memory, Visuospatial/Constructional, Language, Attention and Delayed Memory.

The Immediate Memory index consists of two subtests – List Learning and Story Memory. In List Learning participants were read a list of 10 unrelated words and then asked to recall as many words as they could. This list of words was repeated three more times. The raw score was the total number of recalled words from all four trials with a score range of 0

to 40. In Story Memory participants were read a story with 12 core components and then asked to retell the story from memory. This was repeated once more with the same story. The raw score was the total number of recalled components of the story from both trials with a score range of 0 to 24.

The Visuospatial/Constructional index consists of two subtests – Figure Copy and Line Orientation. In Figure Copy participants were shown a geometric figure and asked to copy it while the figure remained in front of them with a maximum of four minutes to complete the task. The raw score was the total number of correctly drawn and positioned parts of the figure with a score range of 0 to 20. In Line Orientation participants were presented with 10 drawings, each drawing consisted of 13 lines radiating from a single point in a semi-circular shape. These lines were numbered from 1 to 13. Below the drawing were two lines which matched two of the lines in the drawing. Participants were asked to identify the two numbers. Participants had a maximum of 20 seconds to name the two numbers for each drawing. The raw score was the total number of correct responses from all 10 drawings with a score range of 0 to 20.

The Language index consists of two subtests – Picture Naming and Semantic Fluency. In Picture Naming participants were presented with drawings of 10 objects and asked to name each one. Participants were given a semantic clue if the drawing was misidentified. The raw score was the total number of correctly identified drawings with a score range of 0 to 10. In Semantic Fluency participants were asked to name as many examples as possible from a given semantic category such as different kinds of fruits and vegetables or animals found in a zoo. The raw score was the total number of correct responses within 60 seconds with a score range of 0 to 40 for form A, a score range of 4 to 40 for form B and C and a score range of 2 to 40 for form D. The variations in these raw scores was due to adjustments

made to accommodate the level of difficultly of forms B and C (animals you would find in a zoo and musical instruments) by adding four additional points to the raw scores and form D (articles of clothing) by adding two additional points to the raw score.

The Attention index consists of two subtests – Digit Span and Coding. In Digit Span participants were read a string of numbers and then asked to repeat it back. The string of numbers increased by one digit for each trial. The raw score was the total number of correct strings with a score range of 0 to 16. In Coding participants were presented with the back page of the record form which contained rows of boxes with nine different geometric shapes in a random sequence above each box. At the top of the page was a key that consisted of two rows, one row with the nine geometric shapes and the row below with the numbers 1 to 9. Using this key, participants were asked to fill in the number that matched each of the geometric figures. The raw score was the total number of items correctly coded within 90 seconds with a score range of 0 to 89.

The Delayed Memory index consists of four subtests – List Recall, List Recognition, Story Memory and Figure Recall. In List Recall participants were asked to recall words from the 10 words learned in the List Learning subtest. The raw score was the total number of recalled words with a score range of 0 to 10. In List Recognition participants were read 20 words, 10 of which were from the List Learning subtest and 10 were distractor words. After each word was read, participants were asked to indicate whether the word was on the original word list or not. The raw score was the total number of correctly identified words with a score range of 0 to 20. In Story Memory participants were asked to retell the story from the Story Memory subtest. The raw score was the total number of recalled components of the story with a score range of 0 to 12. In Figure Recall participants were

asked to draw from memory the figure from Figure Copy. The raw score was the total number of correctly drawn and positioned parts of the figure with a score range of 0 to 20.

A standardised total RBANS score, five index scores and 12 subtest scores for each form were calculated based on age norms (Randolph, 2012).

7.6.2 National Adult Reading Test – NART

The NART is a test of pre-morbid functioning and was administered only at the baseline testing session. It required participants to read aloud 50 irregular words such as 'Heir' and 'Zealot', printed in two columns. The raw score was the total number of words correctly pronounced. The number of words incorrectly pronounced/number of errors was used to calculate a predicted IQ score: Predicted IQ score = 128 - 0.83 x number of errors.

7.6.3 Coin Rotation Task – CRT

The CRT is a test of fine motor coordination. Participants were asked to position a NZ 20 cent coin in between their middle and index fingers on their dominant hand, and then to flip the coin as many times as they could in 20 seconds. Participants were asked to repeat this task three times and the final raw score was an average of the three trials. Raw scores were converted to z-scores based on age and gender norms (Thornton & Leathem, 2013).

7.6.4 California Older Adult Stroop Test – COAST

The COAST is a test of attention and executive function, specifically the ability to inhibit information. There are three parts in this version of the Stroop, in each part participants were given a laminated A4 sheet to read off. In part one, the page contained blocks of the colours red, green and yellow, and participants were asked to name the blocks

of colours that were arranged on the page in 10 rows of five blocks. Participants were asked to name each block of colours by reading left to right from the top to the bottom of the page as quickly as they could. In part two, the page contained the words of the colours (e.g., RED, GREEN, YELLOW) printed in black ink, again in a 10 by five format. Participants were asked to read the words from top to bottom as quickly as they could. In part three, the page contained the words RED, GREEN and YELLOW printed in either red, green or yellow ink, with the ink colour incongruent to the meaning of the word. Participants were asked to name the colour of the ink that the word was printed in as opposed to reading the word. The main raw score was the time taken to complete part three of the task. The time taken to complete the other two parts of the test as well as the number of self-corrected and non-corrected errors was also measured. Raw scores were converted to z-scores (Pachana et al., 2004).

7.6.5 Trail Making Test A and B – TMT

The TMT is a test of attention and mental flexibility. This test contained two parts, A & B. In part A, participants were presented with an A4 page that contained the numbers 1 to 25 inside small circles which were randomly distributed among the page. Participants were told to join up the numbers in numerical order using a pencil. In part B, participants were presented with an A4 page that contained the numbers 1 to 13 and the letters A through to L, again inside small circles randomly distributed among the page. Participants were told to join up the numbers and letters in an alternating sequence using a pencil. Before each part was administered, a small practice sample was completed to ensure participants understood the task. Participants were told to complete the task as quickly as they could. The raw score was the time taken to complete each part as well as the total number of

errors. Raw scores were converted to percentiles based on norms by age and gender (Shirk et al., 2011).

7.6.6 Digit Span Backward Test – DSBT

The DSBT is a test of working memory. Participants were read a string of digits and were asked to repeat the digits in a backwards sequence. Two string lengths of the digit string were presented and was increased by one extra digit with each trial. The raw score was the total number of correct strings. Raw scores were scaled (Wechsler, 1997) based on age and then converted to percentiles based on norms by age and gender (Shirk et al., 2011). Raw scores from the Digit Span Backward Test and the Digit Span Forward (Digit span from RBANS) were scaled based on age (Wechsler, 1997) to produce both a Digit Span Forward score (DSF) and a Digit Span Total score (DSTot).

7.7 Well-being measures

The well-being measures used were the Geriatric Depression Scale (GDS; Sheikh, & Yesavage, 1986), the Geriatric Anxiety Inventory (GAI; Pachana et al., 2007) and 36-Item Short Form Health Survey Questionnaire (SF-36; Ware & Gandek, 1994).

7.7.1 Geriatric Depression Scale – GDS

The GDS is a self-reported measure of depression. Participants were given an A4 page with 15 yes or no questions. Participants were asked to read each question and then circle ‘Yes’ or ‘No’ to describe whether they felt this way or not over the past week. An example question was ‘Do you feel that your life is empty?’. The raw score was the total number of positive responses, with a score range of 0 to 15. A GDS score between 0 to 4

suggested no indication of depression, a score between 5 to 9 suggested some indication of depression, and 10 or more suggested an indication of depression.

7.7.2 Geriatric Anxiety Inventory – GAI

The GAI is a self-reported measure of anxiety. Participants were given an A4 page with 20 statements. Participants were asked to read each statement and then tick the 'Agree' or 'Disagree' column to reflect how they felt over the past week. An example statement was 'I often feel nervous'. The raw score was the total number of positive responses, with a score range of 0 to 20. A GAI score between 0 to 8 indicated an absence of anxiety and a score of 9 or more indicated the presence of anxiety.

7.7.3 36-Item Short Form Health Survey Questionnaire – SF-36

The SF-36 is a self-reported measure of health and quality of life. The survey consists of 36 questions, with 26 of the questions on a 5-point Likert scale and 10 questions on a 3-point Likert scale. The survey is divided into eight domains and scores: physical functioning, role physical (limitation in usual role due to physical health problems), bodily pain, general health, vitality, social functioning, role emotional (limitation in usual role due to emotional problems) and mental health. Scores were standardised and were based on age norms. A total physical and mental score were also calculated.

7.8 Anthropometric measurements

Weight was measured using a dial scale, and height using a wall-mounted stadiometer. Body mass index (BMI) was calculated by dividing participants' weight in kilograms by height in metres squared. Systolic and diastolic blood pressure were measured

on the non-dominant arm and after the participant had been seated for at least 15 minutes, using an automated sphygmomanometer (Sure Signs VM4; Philips Medical Systems, Andover, MA).

7.9 Biochemical analysis

Participants had two fasting blood samples taken throughout the trial. The first sample was taken after the baseline testing session and was analysed for red blood cell (RBC) fatty acid profile status, vitamin B12 and ferritin levels. The second sample was taken after the final testing session and was analysed for RBC fatty acid profile status and APOE genotyping. Blood samples were taken in one of the Southern Community Laboratories (SCL) collection centres in and around Wellington, including a collection point at the outpatients area of the Wellington Regional Hospital. The Wellington Hospital laboratory prepared, stored and transported the blood samples to the Nutrition Laboratory at the Institute of Food, Nutrition and Human Health, Massey University in Palmerston North for RBC fatty acid status, and Canterbury Health Laboratories (CHL) in Christchurch for APOE genotyping. Wellington regional hospital laboratories ran the analysis for vitamin B12 and ferritin status. All three laboratories have International accreditation. All blood samples were destroyed once analysed.

7.9.1 Red blood cell fatty acid profile status

This test measured erythrocyte or RBC levels of 38 fatty acids including LA, ALA, ARA, EPA, DPA and DHA with a unit measure of mg/mL as well as total % fatty acids. Values of the EPA and DHA levels were used to calculate an omega-3 index score: omega-3 index = EPA +

DHA / total % fatty acids. Values were also used to calculate two *n*-6:*n*-3 ratios – ARA:EPA and ARA:DHA.

Blood samples were collected in 5 mL lithium/heparin tubes and centrifuged at 3000 x g for 10 minutes. The RBCs were then transferred to a cryotube and 1.7 mL butylated hydroxytoluene/methanol was added. The sample was then agitated until homogenous and stored at -80°C. Per 50 uL RBCs, 200 uL of internal standard working solution was added. The internal standard (IS) stock solution consisted of 22.5 mg +/-0.5 mg of heptadecanoic acid dissolved in 100 mL of methanol. The IS solution was diluted further to 22.5 ug/mL by adding 2500 uL to 25 mL of methanol. Per 50 uL RBCs, 1000 uL of 3N methanolic hydrochloric acid was added to each sample tube. The samples were incubated for 4 hours at 90˚C with occasional mixing, and then left to cool to room temperature. Samples were transferred into kimax tubes and per 50 uL RBCs a further 2000 uL of hexane was added to each sample. Samples were shaken manually for 10 seconds, vortexed for 1 minute and then centrifuged at 2000 rpm and 20˚C for 3 minutes. The top layer was removed and transferred to round bottom flask. Samples were dried with a rotary evaporator at 38˚C. Samples were dissolved by adding 100 uL of hexane per 50 uL RBCs to each tube, using a calibrated pipette. Samples were then transferred to gas chromatography vials. Fatty acid analysis was run using a gas chromatograph (Shimadzu G-2010 plus). The concentration of fatty acid of each sample was calculated by comparing the average area of the sample to the concentration and area of the international standard in the standard solution.

7.9.2 Vitamin B12 and Ferritin

Low levels of vitamin B12 and ferritin have been associated with cognitive decline in older adults (Morris et al., 2005b; Morris et al., 2007), and therefore these two nutrient

levels were measured to rule out the effect of a deficiency or a secondary cause for any cognitive impairment (Blennow et al., 2006). Vitamin B12 was measured by pmol/L units with a reference range of between 170 and 800 pmol/L. Ferritin was measured by µg/L units with a reference range of 20 and 350 µg/L.

7.9.3 APOE genotyping

APOE genotyping was performed using a Polymerase Chain Reaction in a DNA Thermal Cycler (Perkin Elmer Cetus) using the method described by Hixson and Vernier (1990). The results of the APOE test comprised of two APOE alleles/numbers (e.g., Apolipoprotein E genotype: E3/4) for each participant.

7.10 Questionnaire

A list of the participant's medications and supplements, existing health conditions including any vision or hearing impairments and family history of dementia or memory problems was compiled at the baseline session along with general demographic information including date of birth, country of birth, relationship status, ethnicity, employment status, occupation (including prior occupation if retired), income level, years of education, highest qualification obtained, number of languages spoken, left or right handed, frequency of computer use, and other health related behaviours such as cigarette smoking – both current and past, weekly alcohol consumption, average number of hours sleep per night and amount of weekly exercise and weekly mental engagement. At the 2nd and 3rd testing sessions, participants were asked about changes in medication and supplement use, the number of hours sleep per night, weekly exercise and mental engagement. At the final testing session participants were asked which type of capsule they thought they had been

given during the trial, and also whether they thought their memory and cognition had improved, declined or remained the same throughout the trial.

7.11 Nutritional status questionnaire

Participants completed a nutritional screening questionnaire during the baseline testing session. This questionnaire was designed to screen out participants who consumed more than two servings of fish and other seafood per week. See Appendix J for the nutritional screening questionnaire. The first question asked whether the participant consumed any type of fish or seafood. If the answer was 'yes' the participant was asked how many servings of fish and seafood were consumed per week and then asked to specify how often they consumed certain types of fish and seafood. Participants were asked to limit consumption of fish and seafood to two servings per week, and to maintain their usual diet throughout the course of the trial.

7.12 Calendar

Participants were given a 12-month calendar at the end of the baseline testing session and were asked to answer three daily 'Yes' or 'No' questions on the calendar during the 12-month supplement period. Each day participants were asked to circle 'Y' or 'N' for the following three questions: Taken pills Y/N, Ate fish Y/N, Felt unwell Y/N. If participants circled 'Y' for 'Ate fish' they were asked to write down on the calendar what type of fish they had eaten. The 'Taken pills' question was part of the compliance procedure.

7.13 Procedure

Participants completed the anthropometric, cognitive and well-being measures for all four testing session in the same order. All testing sessions, except the sessions for three participants, took place at the Massey University Psychology Clinic at 24 King Street, Mount Cook in Wellington. For two of the three participants, all four testing sessions took place in the participant's home and for the fourth participant the first three testing sessions took place at the Psychology clinic and the final testing session in the participant's home. Participants were reimbursed for participating in this study with a $20 supermarket voucher for each testing session.

7.13.1 Baseline testing session

The first testing session was divided into four parts. The first part involved explaining the time frame of the trial, giving the participant a copy of the signed consent form, administering the baseline questionnaire, and then measuring blood pressure, weight and height. This took between 30 and 45 minutes. The second part involved the cognitive measures in the following order: NART, RBANS version A, CRT, COAST, DSBT and TMT. This took between 45 minutes to 1 hour. Participants were then given a 15-minute break. During the break participants had a refreshment of a hot or cold drink and biscuit/cookie, and then took a toilet break. In the third part participants completed the GDS, GAI, SF-36 and the Nutritional Screening Questionnaire. This took approximately 20 minutes. The fourth part involved explaining the first blood test and the patient form, dispensing the first four bottles of capsules and explaining how and when to take the capsules, explaining how to use the calendar, and finally handing over the supermarket voucher. This took approximately 20 minutes.

7.13.2 Second testing session

The second testing session was divided into four parts. The first part involved the questionnaire, and then measuring blood pressure, weight and height. This took approximately 20 minutes. The second part involved the cognitive measures in the following order: RBANS version B, CRT, COAST, DSBT and TMT. This took between 40 minutes and 1 hour. Participants were then given a 5-minute break. In the third part participants completed the GDS, GAI and SF-36. This took approximately 15 minutes. The fourth part involved counting the returned capsules, and handing over the 5th bottle of capsules and supermarket voucher. This took approximately 10 minutes.

7.13.3 Third testing session

The third testing session was divided into four parts. The first part involved the questionnaire, then measuring weight and height. This took approximately 15 minutes. The second part involved the cognitive measures in the following order: RBANS version C, CRT, COAST, DSBT and TMT. This took between 40 minutes and 1 hour. Participants were then given a 5-minute break. In the third part participants completed the GDS, GAI and SF-36. This took approximately 15 minutes. The fourth part involved counting the returned capsules, handing over the 6th bottle of capsules and the supermarket voucher. This took approximately 10 minutes.

7.13.4 Final testing session

The final testing session was also divided into four parts. The first part involved the final questionnaire, and then measuring blood pressure, weight and height. This took approximately 30 minutes. The second part involved the cognitive measures in the following

order: RBANS version D, CRT, COAST, DSBT and TMT. This took between 45 minutes and 1 hour. Participants were then given a 5-minute break. In the third part participants completed the GDS, GAI and SF-36. This took approximately 20 minutes. The fourth part involved counting the returned capsules, completing the final summary questionnaire, explaining the dissemination of the results and the handing out the final supermarket voucher. This took approximately 15 minutes.

7.14 Storage of information and participant feedback

All participant information collected during and after the trial was stored in a locked filing cabinet and on a password-locked computer.

All participants answered 'Yes' to receiving individual and grouped cognitive results and so at the conclusion of the trial all participants were sent both an individual results report and grouped results summary page. See Appendix K and L. Results of the vitamin B12, ferritin and RBC fatty acid profiles were given over the phone. Copies of the results were forwarded if requested. If a participant indicated on the consent form that they wanted to know the results of the APOE genotyping test, the individual report as provided by CHL was sent to the participant via the post. If the participant was an APOE ε4 allele carrier, they were also sent a copy of the APOE fact sheet (see Appendix M). If a participant then requested further information about any of the biochemical tests, they were advised to contact their nurse or General Practitioner.

7.15 Data analysis

Differences between participant completers and drop-outs, and between the two groups at baseline were analysed using two-tailed independent samples *t*-tests and chi-

square tests. ANCOVAs were used to test for treatment effects on the measures, with treatment group as the main factor and with age, gender, and years of education as covariates. All assumptions were met for the linear model analyses including Levene's test of equality of error variances. Bonferroni corrections were used for multiple analyses. The alpha was set at 0.05 for all analyses, all of which were performed using IBM SPSS statistics program version 25. Cohen's *d* values were calculated using the formula $d = t\sqrt{\frac{1}{n1} + \frac{1}{n2}}$ from an effect size calculator, where an effect size of 0.2 was considered small, 0.5 was medium and 0.8 was large (Lakens, 2013). Eta squared effect sizes were calculated with SPSS using the formula $\eta^2 =$ SS effect / SS total, where .01 was considered small, .09 was medium and .25 was large.

CHAPTER 8

RESULTS

8.1 Participant completers and drop-outs

Seventy-two participants were enrolled in the 12-month RCT. Participants were randomly assigned to receive either the DHA supplement (*n* = 37) or a placebo (*n* = 35). Of the 72 participants, 12 withdrew (seven from the DHA group and five from the placebo group). One participant died (placebo) between the 2nd and 3rd testing sessions due to a pre-existing health condition, but otherwise all participant drop-outs occurred voluntarily between the baseline and 2nd testing sessions. See Figure 5 for a flow diagram showing participant drop-outs and completers. The 12 participant drop-outs resulted in an attrition rate of 17%. There were no demographic, cognitive or well-being differences between participants who dropped out and completed the trial. The final analysis was performed with a total of 60 participants – 30 participants in each group.

8.2 Baseline characteristics of the participants

The baseline characteristics of participants in the DHA and placebo groups are summarised in Table 11. There were no differences between the two groups in any of the demographic variables or in any of the cognitive and well-being measures at baseline.

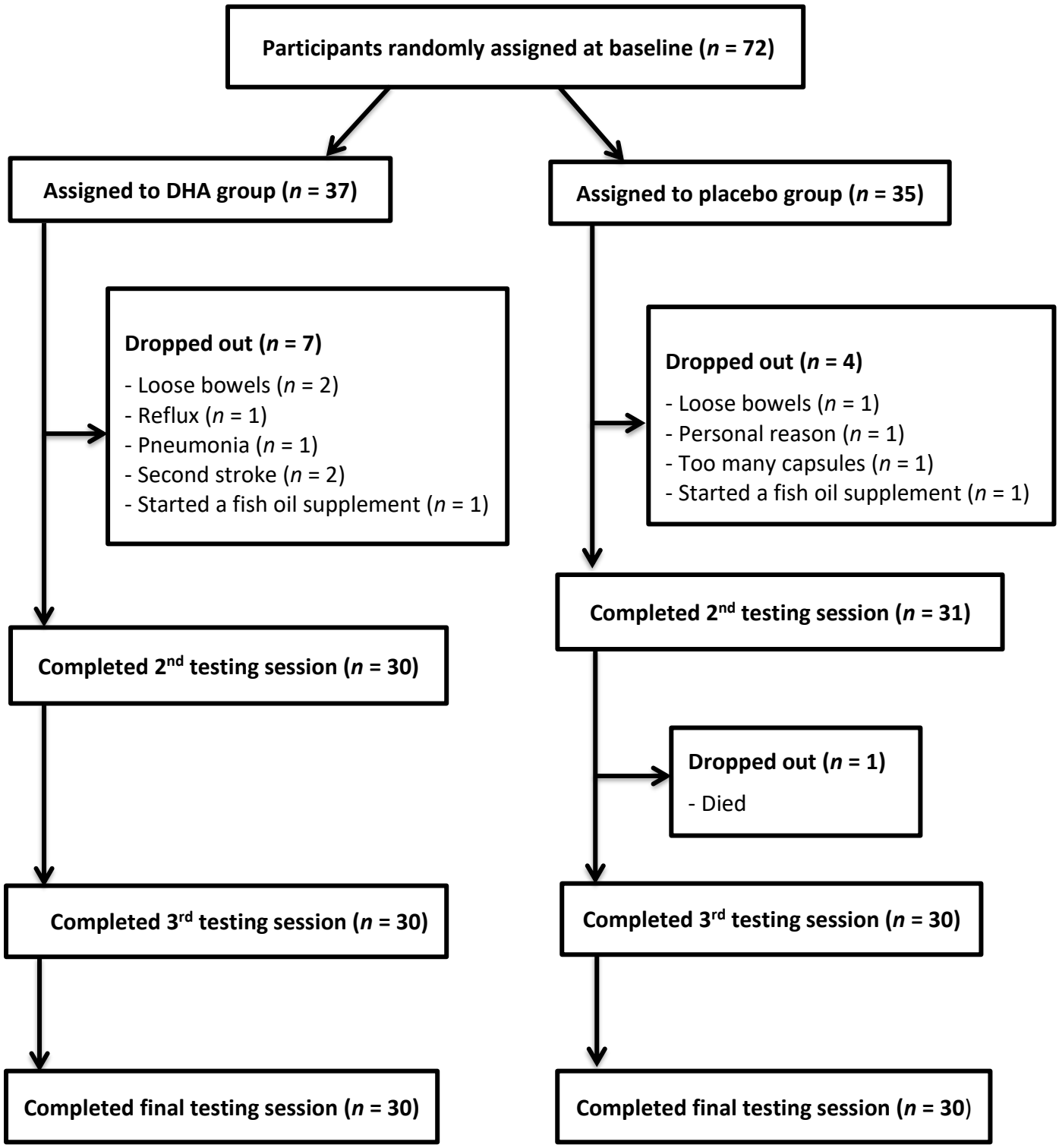

Figure 5. Participant drop-outs and completers

The NART predicted IQ scores were more than 1 SD above standardised average IQ, indicating that the participant group was above average in IQ and met the MCI criteria for preserved general cognition/IQ. The only significant difference between the two groups at baseline was in ARA levels, with a significantly higher baseline ARA level for the placebo group (M = 19.99, SD = 1.83) compared to the DHA group (M = 18.87, SD = 1.64), $t(58)$ = 2.49, p = .02, d = 0.63.

At baseline, women (M = 56.18, SD = 6.81) scored significantly higher than men on the SF-36 general health score (M = 52.06, SD = 7.65), $t(58)$ = -2.19, p = .03, d = 0.57 and had significantly higher EPA (women M = 1.10, SD = 0.51; men M = 0.86, SD = 0.37), $t(58)$ = -2.03, p = .04, d = 0.53 and DHA levels (women M = 4.35, SD = 0.83; men M = 3.73, SD = 1.07), $t(58)$ = -2.52, p = .02, d = 0.63. At baseline there was also a trend towards men (M = 0.05, SD = 1.31) scoring higher than women (M = -0.54, SD = 1.00), $t(58)$ = 1.97, p = .05, d = 0.52 on the Coin Rotation Task.

Of the 60 participants, 30% were carriers of APOE ε4 (5 ε2/ε4, 12 ε3/ε4 and 1 ε4/ε4) and 27% were carriers of APOE ε2 (0 ε2/ε2 carriers, 11 ε2/ ε3 and 5 ε2/ε4). APOE ε4 carriers had significantly higher baseline GDS scores (M = 3.00, SD = 2.17) than non-ε4 carriers (M = 1.67, SD = 2.07), $t(58)$ = -2.26, p = .03, d = 0.62, and higher baseline GAI scores (M = 3.61, SD = 4.34) than non-ε4 carriers (M = 1.90, SD = 2.68), $t(58)$ = -1.86, p = .07, d = 0.53. APOE ε4 carriers had lower baseline SF-36 Role physical scores (M = 47.68, SD = 7.45) than non-ε4 carriers (M = 51.34, SD = 5.93), $t(58)$ = 2.03, p = .05, d = 0.56, and lower baseline SF-36 Physical functioning scores (M = 47.68, SD = 8.45) than non-ε4 carriers (M = 51.93, SD = 4.95), $t(58)$ = 2.44, p = .02, d = 0.68. There were no differences between ε4 carriers and non-ε4 carriers in the demographic or cognitive measures at baseline.

Table 11. Baseline characteristics of DHA and placebo groups

Characteristic	DHA group (*n* = 30)	Placebo group (*n* = 30)	*P*	*d*
Age (years)	72.33 (6.16) [1]	73.40 (6.96)	.53	0.16
Gender (Male/Female)	14/16	11/19	.43	
NZ born – Euro/Pakeha (%)	73	83	.67	
Handed (L/R)	3/27	7/23	.16	
Married (%)	47	37	.09	
Education (years)	15.56 (2.83)	14.30 (3.19)	.11	0.39
NART predicted IQ score	119.72 (4.76)	119.02 (5.03)	.58	0.14
BMI score (kg/m^2)	27.54 (4.16)	26.01 (3.94)	.15	0.42
Systolic BP (mmHg)	145.90 (15.98)	140.23 (21.91)	.26	0.29
Diastolic BP (mmHg)	83.67 (11.87)	80.07 (13.77)	.28	0.28
Current smoker (Y/N)	0/30	2/28	.15	
Alcohol (drinks/week)	5.36 (5.87)	4.88 (4.47)	.72	0.08
Sleep (hrs/night)	7.11 (0.96)	6.70 (1.16)	.13	0.38
Exercise (days/week)	4.17 (1.39)	4.53 (1.17)	.28	0.28
Number of med (per day)	2.26 (1.62)	2.0 (1.52)	.46	0.19
Hypertension med (Y/N)	16/14	17/13	.80	
APOE ε4 carriers (n/%)	10/33	8/27	.58	
APOE ε2 carriers (n/%)	7/23	9/30	.57	
LA (%/TFA)	7.96 (1.03)	7.95 (1.32)	.94	0.01
ARA (%/TFA)	18.87 (1.64)	19.99 (1.83)	.02*	0.64
EPA (%/TFA)	1.00 (0.50)	0.99 (0.44)	.95	0.02
DPA (%/TFA)	1.9 (0.35)	1.85 (0.31)	.48	0.15
DHA (%/TFA)	4.1 (1.01)	4.06 (0.96)	.84	0.04
Omega-3 index	5.12 (1.29)	5.06 (1.11)	.94	0.02
Vitamin B12 (pmol/L)	284.67 (112.08)	325.86 (232.08)	.39	0.22
Ferritin (µg/L)	182.40 (152.36)	150.30 (115.03)	.36	0.24

Note. NZ = New Zealand, Euro = European, NART = National adult reading test, BMI = Body mass index, BP = Blood pressure, med = medication, APOE = Apolipoprotein, LA = Linoleic acid, TFA = total % fatty acid, ARA = Arachidonic acid, EPA = Eicosapentaenoic acid, DPA = Docosapentaenoic acid, DHA = Docosahexaenoic acid, Omega-3 index = EPA + DHA / total % fatty acid.

[1] Values are means; SDs in parentheses * = $p < 0.05$

8.3 Primary outcome measures

The primary outcome was a change in the cognitive, well-being and anthropometric measures from baseline to 12 months. Table 12 shows that the only significant treatment effect was on systolic blood pressure, where systolic blood pressure increased more in the placebo group (*Mdiff* = 10.70, *SD* = 14.53) over the 12-month period than in the DHA group (*Mdiff* = 1.83, *SD* = 16.23) $F(1, 55) = 4.97$, $p = .03$, $\eta^2 = .08$. The change in scores from baseline to 6-months in the cognitive, well-being and anthropometric measures were also analysed but showed no treatment effects. See Appendix N for the mean scores of the two groups at baseline, 6-months and 12-months and Appendix O for change in measures by group from baseline to 6 months. The data from the 9-month testing session was excluded because several participants were unavailable for testing due to unforeseen circumstances (e.g., illness, work commitments, and need to travel overseas for family reasons).

8.4 Secondary outcome measures

The secondary outcome was the treatment x APOE interaction effect on the change in cognitive, well-being and anthropometric measures. There was a significant effect for APOE ε4 x treatment on GDS and GAI scores, see Figures 6 and 7. GDS scores significantly improved in ε4 carriers in the DHA group compared to ε4 carriers in the placebo group $F(1, 53) = 4.16$, $p = .04$, $\eta^2 = .07$, and GAI scores significantly improved in ε4 carriers in the DHA group compared to ε4 carriers in the placebo group $F(1, 53) = 5.41$, $p = .02$, $\eta^2 = .09$. GDS and GAI scores were correlated ($r = .56$, $p < .01$) across all participants.

Table 12. Change in cognitive, well-being and anthropometric measures by group from baseline to 12 months

	DHA group (*n* = 30)			Placebo group (*n* = 30)			DHA compared to Placebo				
	Baseline	12 months	Change	Baseline	12 months	Change	Difference	Treatment effect		APOE ε4 x treatment	
								p	η^2	*p*	η^2
RTot	101.9 (11.9)[1]	108.4 (12.8)	6.5 (5.8)	100.3 (11.9)	105.5 (11.2)	5.2 (7.4)	1.3 (-4.7, 2.1)[2]	.37[3]	.02	.64	.00
RIM	104.8 (16.2)	110 (13.6)	5.2 (8.7)	105.3 (13.9)	109.9(13.8)	4.5 (12.3)	0.6 (-6.1, 4.8)	.68	.01	.66	.00
RVC	96.6 (9.6)	98.4 (8.8)	1.7 (9.1)	92.9 (12.6)	91.4 (11.8)	-1.4 (13.1)	3.1 (-9.0, 2.7)	.28	.02	.99	.00
RL	103.9 (13.8)	107.0 (101.1)	3.06 (13.1)	103.2 (12.8)	106.7 (7.8)	3.5 (11.7	-0.4 (-5.9, 6.8)	.86	.00	.83	.00
RA	103.7 (13.8)	110.4 (15.3)	6.7 (8.1)	102.9 (13.3)	109.8 (13.9)	6.9 (10.5)	-0.2 (-4.6, 5.0)	.93	.00	.73	.00
RDM	98.2 (11.3)	104.6 (11.9)	6.3 (7.9)	98.3 (12.7)	102.8 (12.9)	4.5 (10.3)	1.8 (-6.5, 2.9)	.45	.01	.38	.01
CRT	-0.33 (1.2)	-0.003 (1.4)	0.33 (0.7)	-0.25 (1.2)	-0.12 (1.5)	0.13 (0.96)	0.1 (-0.6, 0.24)	.38	.01	.50	.01
COA	57.8 (22.9)	65.2 (21.5)	7.4 (16.5)	53.7 (22.8)	64.8 (22.4)	11.1 (13.3)	-3.6 (-4.1, 11.4)	.35	.02	.23	.03
DSF	75.4 (24.3)	84.5 (19.1)	9.1 (20.9)	63.2 (26)	77.1 (22.1)	13.9 (24)	-4.7 (-6.8, 16.4)	.42	.01	.58	.01
DSBT	56.1 (32.5)	63.6 (31.1)	7.6 (17.3)	49.5 (30.5)	51.4 (30.1)	1.86 (24.5)	5.7 (-16.6, 5.3)	.30	.02	.56	.01
DSTot	12.4 (3.4)	13.6 (3.4)	1.2 (2.3)	11.2 (3.2)	12.1 (3)	0.9 (2.2)	0.3 (-1.4, 0.8)	.60	.01	.40	.01
TMTB	41.9 (30.4)	48.0 (31.1)	6.1 (26.2)	49.8 (31.6)	56.8 (32.3)	6.9 (37)	-0.8 (-15.7, 17.4)	.91	.00	.81	.00
GDS	1.97 (2)	1.97 (2.7)	0.0 (1.4)	2.2 (2.4)	2.1 (2.2)	0.03 (2.3)	-0.03 (-0.9, 1.0)	.95	.00	.04*	.07
GAI	2.2 (3.0)	1.83 (3.1)	0.4 (2.4)	2.6 (3.6)	1.9 (2.4)	0.7 (2.6)	-0.3 (-0.9, 1.6)	.64	.00	.02*	.09
SF36PF	50.0 (6.9)	50.0 (6.6)	0.03 (2.9)	51.3 (5.9)	49.8 (6.4)	-1.5 (4)	1.5 (-0.2,3.3)	.09	.05	.83	.00
SF36BP	50.5 (7.7)	52.1 (7.4)	1.6 (8.9)	52.6 (8.8)	49.9 (8.1)	-2.6 (11.1)	4.2 (-0.95, 9.5)	.09	.05	.58	.01
SF36SF	52.3 (6.7)	53.5 (5.7)	1.17 (6.3)	51.6 (6)	49.9 (9.3)	-1.5 (9.6)	2.6 (-1.5, 6.8)	.21	.03	.71	.00
SBP	145.9 (15.9)	147.7 (16.7)	1.8 (16.2)	140.2 (21.9)	150.9 (25.1)	10.7 (14.5)	8.8 (-0.9, 16.8)	.03*	.08	.47	.01
DBP	83.6 (11.9)	84 (12.9)	0.3 (10.9)	80.1 (13.7)	81.9 (12.1)	1.83 (10)	1.5 (-3.9, 6.9)	.57	.01	.35	.02

Note. RTot = RBANS Total score, RIM = RBANS Immediate Memory, RVC = RBANS Visuospatial/Constructional, RL = RBANS Language, RA = RBANS Attention, RDM = RBANS Delayed Memory, CRT = Coin Rotation Task, COA = COAST incongruent score, DSF = Digit Span Forward, DSBT = Digit Span Backward Test, DSTot = Digit Span Total, TMTB = Trail Making Test B, GDS = Geriatric Depression Scale, GAI = Geriatric Anxiety Inventory, SF36PF = SF-36 Physical functioning, SF36BP = SF-36 Bodily pain, SF36SF = SF-36 Social functioning, SBP = Systolic blood pressure, DBP = Diastolic blood pressure

[1] Values are means; SDs in parentheses [2] Values are means; 95% CIs in parentheses [3] *P* values were derived by using ANCOVA (adjusted for age, gender and years of education) with APOE as an independent variable to test APOE ε4 x treatment interaction. None of the analyses violated Levene's test of equality of error variances.

* = $p < 0.05$

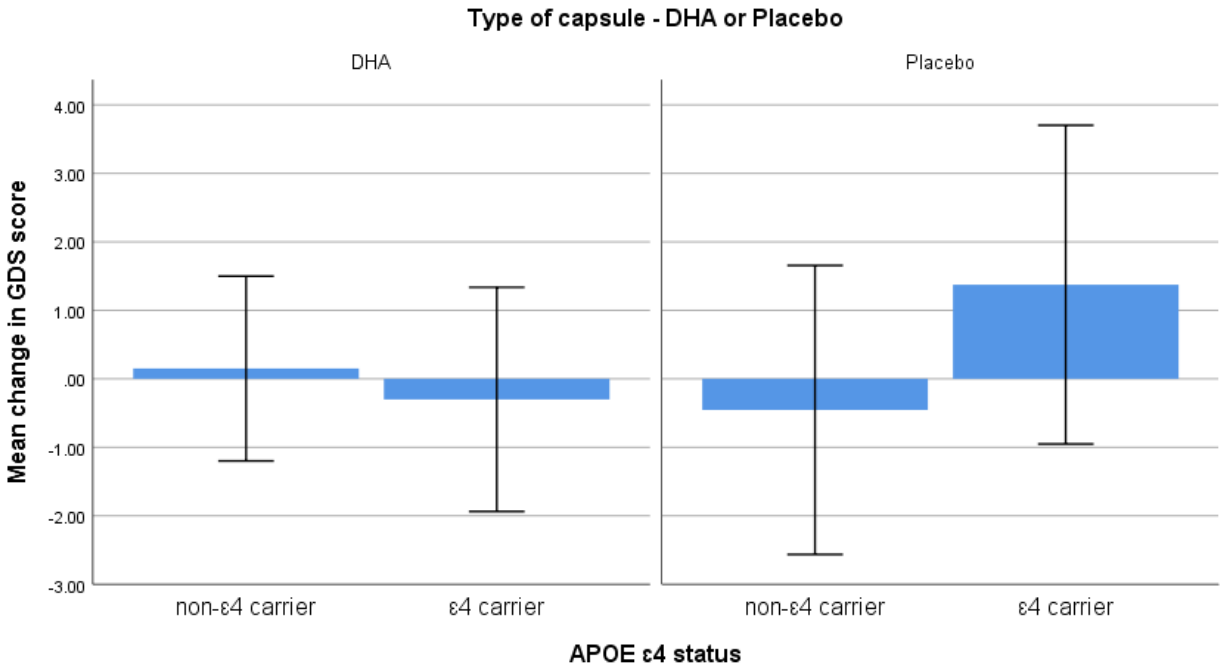

Figure 6. Mean change in GDS scores from baseline to 12-months in APOE ε4 carriers and non-ε4 carriers

Note. Values are means with 1 standard deviation represented by vertical bars. A decrease in score indicates an improvement.

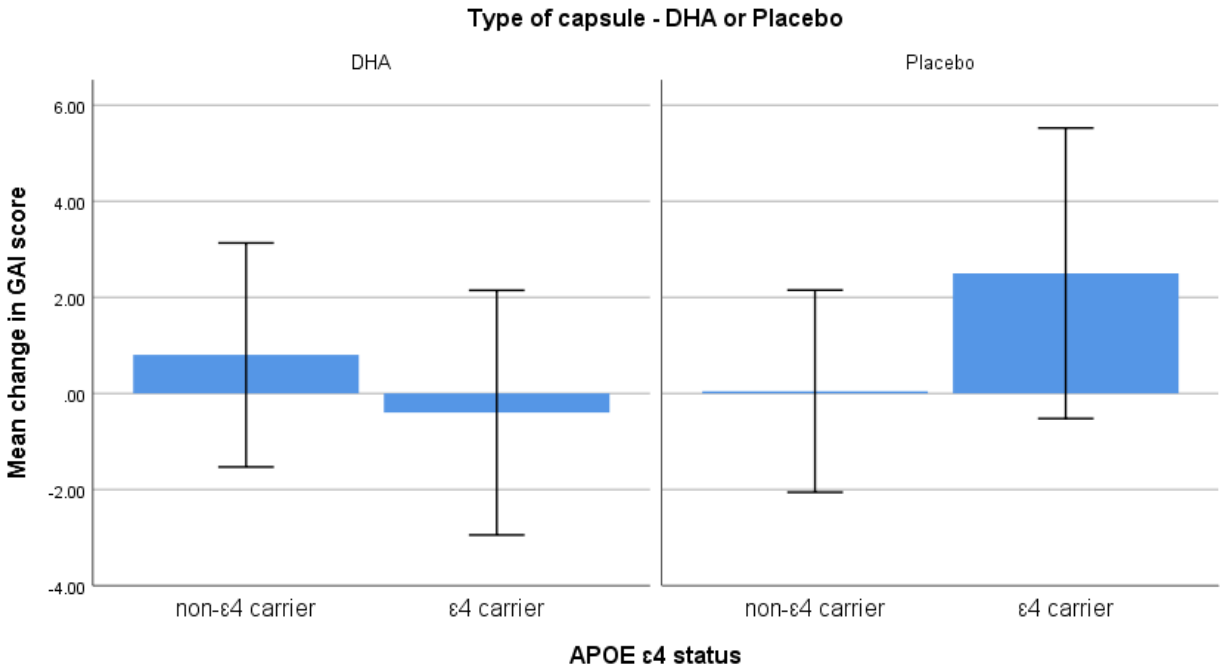

Figure 7. Mean change in GAI scores from baseline to 12-months in APOE ε4 carriers and non-ε4 carriers

Note. Values are means with 1 standard deviation represented by vertical bars. A decrease in score indicates an improvement.

There were no other APOE ε4 x treatment effects on the cognitive and well-being measures. There was a significant effect for APOE ε2 x treatment on RBANS Total scores, in that RBANS Total scores were significantly improved in APOE ε2 carriers in the placebo group (*Mdiff* = 9.57, *SD* = 10.29) compared to APOE ε2 carriers in the DHA group (*Mdiff* = 4.00, *SD* = 6.32) $F(1, 52) = 7.04$, $p = .01$, $\eta^2 = .12$. There were no gender x treatment effects, nor any age x treatment effects in any of the cognitive or well-being measures. Overall, RBANS immediate memory scores improved significantly more in non-ε4 carriers (*M* = 6.90, *SD* = 10.55) than in ε4 carriers (*Mdiff* = 0.11, *SD* = 9.26), $F(1, 55) = 5.18$, $p = .03$, $\eta^2 = .09$, and RBANS delayed memory score improved significantly more in non-ε4 carriers (*Mdiff* = 7.33, *SD* = 8.37) than in ε4 carriers (*Mdiff* = 0.88, *SD* = 9.54), $F(1, 55) = 6.49$, $p = .01$, $\eta^2 = .11$. There were no other differences between ε4 carriers and non-ε4 carriers in any of the measures.

8.5 Compliance, seafood consumption and fatty acid levels

Compliance rates at the end of the trial were on average 93% across the groups, with no significant difference between the DHA group (*M* = 92.51, *SD* = 8.14) and the placebo group (*M* = 94.79, *SD* = 5.25), $t(58) = 1.27$, $p = .21$, $d = 0.32$. This compliance was confirmed by changes in RBC fatty acids levels (see Table 13). RBC fatty acid levels of EPA ($d = 1.00$), DHA ($d = 1.76$) and total *n*-3 ($d = 1.39$) increased in the DHA group compared to the placebo group (all $p < .01$), and both the ARA:EPA ($d = 1.07$) and ARA:DHA ($d = 1.68$) ratios decreased in the DHA group compared to the placebo group (both $p < .01$).

Table 13. Change in PUFA levels of DHA and placebo groups from baseline to 12 months

	DHA group (*n* = 30)			Placebo group (*n* = 30)			Difference		
PUFA	Baseline	12 months	Change	Baseline	12 months	Change	DHA to Placebo	*P*	*d*
LA	7.97 (1.06)[1]	7.41 (1.04)	-0.55 (0.87)	7.95 (1.32)	7.78 (1.53)	-0.16 (0.99)	-0.39 (-0.87, -0.09)[2]	.12	0.42
ALA	0.00	0.00	0.00	0.08 (0.21)	0.00	-0.08 (0.21)	0.08 (0.004, 0.15)[3]		
ARA	18.87 (1.64)	13.32 (2.88)	-5.55 (2.91)	19.99 (1.83)	15.21 (3.78)	-4.78 (3.81)	-0.77 (-0.98, 2.52)	.38	0.20
NA	4.44 (0.84)	5.54 (0.77)	1.09 (1.02)	4.40 (0.96)	5.51 (0.70)	1.11 (0.92)	0.02 (-0.48, 0.52)	.94	0.19
EPA	1.00 (0.50)	1.10 (0.49)	0.09 (0.61)	0.99 (0.44)	0.56 (0.32)	-0.44 (0.47)	0.54 (0.26, 0.82)	<.01**	1.00
DPA	1.92 (0.35)	1.44 (0.43)	-0.48 (0.50)	1.85 (0.31)	1.54 (0.53)	-0.32 (0.65)	-0.16 (-1.14, 0.46)	.29	0.28
DHA	4.11 (1.01)	6.24 (1.78)	2.13 (1.87)	4.06 (0.96)	3.4 (1.36)	-0.66 (1.19)	2.79 (1.97, 3.59)	<.01**	1.76
ARA:EPA	22.87 (9.47)	12.59 (5.37)	-8.95 (10.30)	24.07 (10.18)	26.41 (7.95)	2.32 (10.57)	-11.25 (-16.8, -5.73)	<.01**	1.07
ARA:DHA	4.81 (1.04)	2.27 (0.70)	-2.54 (1.15)	5.21 (1.40)	5.03 (1.92)	-0.17 (1.63)	-2.33 (-3.09, -1.63)	<.01**	1.68
Omega-3	5.12 (1.29)	7.34 (2.20)	2.22 (2.29)	5.06 (1.11)	3.96 (1.56)	-1.10 (1.47)	3.38 (2.39, 4.37)	<.01**	1.72
Total *n*-3	7.04 (1.29)	8.78 (2.50)	1.74 (2.61)	7.00 (1.22)	5.50 (2.01)	-1.49 (1.98)	3.24 (2.05, 4.44)	<.01**	1.39

Note. LA = Linoleic acid, ALA = Alpha-linolenic acid, ARA = Arachidonic acid, NA = Nervonic acid, EPA = Eicosapentaenoic acid, DPA = Docosapentaenoic acid, DHA = Docosahexaenoic acid, ARA:EPA = Ratio of Arachidonic acid to Eicosapentaenoic acid, ARA:DHA = Ratio of Arachidonic acid to Docosahexaenoic acid, Omega-3 = EPA + DHA / total % fatty acid, Total *n*-3 = ALA + EPA + DPA + DHA / total % fatty acid.

[1] Values are means (as percentage of fatty acids); SDs in parentheses

[2] Values are means; 95% CIs in parentheses

[3] Unable to calculate *p* value due to zero values

** = $p < .01$

APOE ε4 carriers had a higher baseline DPA level (*M* = 2.02, *SD* = 0.32) than non-ε4 carriers (*M* = 1.83, *SD* = 0.32), *t*(58) = -2.16, *p* = .04, *d* = 0.63. There were no other differences between ε4 carriers and non-carriers in any of the baseline RBC fatty acid levels or in any changes in fatty acid levels. During the course of the 12-month period seafood consumption was on average 1.14 servings per week across the groups (0.24 servings/week from oily fish) with no significant differences between the DHA group (*M* = 1.08, *SD* = 0.49) and the placebo group (*M* = 1.19, *SD* = 0.57), *t*(58) = 0.75, *p* = .45, *d* = 0.21. Increased baseline levels of DHA (*r* = .33, *p* < .05) and the omega-3 index (*r* = .32, *p* < .05) were associated with baseline weekly fish consumption. Decreased baseline levels of ARA:DHA ratios (*r* = -.39, *p* < .01) were associated with baseline weekly fish consumption.

8.6 Blinding and adverse effects

Over 75% of participants stated they had 'no idea' which type of capsule they had been given during the 12-month supplement period, 66.6% (*n* = 20) from the DHA group and 86.6% (*n* = 26) from the placebo group. In the DHA group 23.3% (*n* = 7) of participants correctly guessed they had been given the DHA capsule, and in the placebo group 10% (*n* = 3) correctly guessed they had been given the placebo capsule. Two participants reported tasting an orange flavour (one DHA, one placebo). The capsules were well tolerated, except for a one gastro-intestinal related complaint in the DHA group.

CHAPTER 9

DISCUSSION

The research described in this thesis aimed to answer four questions: Can a high DHA fish oil supplement improve cognitive performance in older adults with Mild Cognitive Impairment (MCI)? Does the APOE ε4 allele have a moderating effect on cognition and well-being? How popular are fish oil supplements within New Zealand (NZ)? What health behaviours are associated with fish oil supplement use? The first two questions were answered by way of a randomised controlled trial (RCT), and the other two questions through an online cross-sectional survey. This chapter will discuss the results of the RCT and survey as well as implications for the use of fish oil supplements and for dietary intake of *n*-3 PUFAs in preventing cognitive decline in older adults. The chapter will end by discussing the strengths and limitations of the research, and future recommendations.

9.1 The randomised controlled trial

The results of the RCT found that a high DHA fish oil supplement did not improve cognitive performance in older adults with MCI compared to a placebo of sunflower oil. This is the first fish oil supplement trial in older adults with MCI that did not find a significant difference between treatment groups in any of the cognitive measures. The RCT did not find

an improvement in the cognitive measures, despite a significant increase in DHA and EPA levels in the DHA group compared to the placebo group.

The current RCT did find a treatment effect on systolic blood pressure after 12 months. Systolic blood pressure increased by only 1.83 mmHg units in the DHA group compared to an increase of 10.7 mmHg units in the placebo group, indicating that the DHA group showed a significantly smaller increase (a stabilising effect) in systolic blood pressure over the 12-month period. Systolic blood pressure refers to the amount of pressure within the arteries while the heart is contracting, and an increase is related to an increase in artery stiffness which is common with age. Systolic blood pressure in older adults is often used as an indication of the risk of having a stroke or a heart attack. The proposed mechanisms by which *n*-3 PUFAs, EPA and DHA reduce blood pressure are via prostaglandins that cause vasodilation, anti-platelet aggregation and control sodium and water retention (Das, 2001). The link between diet and hypertension is well established (e.g., Schwingshackl & Hoffmann, 2015), which has led to recommendations of eating a diet high in vegetables and fruit, whole grains and starchy vegetables, with legumes, eggs and oily fish as the preferred forms of protein along with regular physical activity and not smoking (Heart foundation, 2016).

A meta-analysis of 31 fish oil RCTs specifically investigating the effects on blood pressure found that reduction in blood pressure was dose-responsive and was strongest in those with hypertension (Morris, Sacks, & Rosner, 1993). The mean systolic blood pressure of the participants in the current RCT at baseline was 143 (indicative of high blood pressure), whereas the average diastolic blood pressure was 81 (normal blood pressure), which backs these findings. This positive effect on blood pressure supports the two fish oil trials by Nilsson et al. (2012) that found a significant reduction in systolic blood pressure in participants with an average systolic blood pressure of 133 which is also considered ‘high’,

and by Witte et al. (2013) who found a significant reduction in diastolic blood pressure in participants with an average diastolic blood pressure of 87.5 and an average systolic blood pressure of 140. The omega-3 index has been used to indicate risk for coronary heart disease with scores less than '4' indicating a high risk, '4-8' indicating an intermediate risk and scores above '8' a low risk (Harris, 2008). The participant group showed a mean baseline omega-3 index score of 5.09 indicating an intermediate risk of heart disease, and after 12-months of supplementation the DHA group was still below a score of '8'. This indicates that a longer period or higher dose of supplementation may have been required to reach the highest level of cardio-protection.

Eighty percent of participants in the current RCT took prescription medications on a daily basis, and 55% of participants were taking at least one type of hypertension medication. The trial by Yurko-Mauro et al. (2010) found that 50% of participants were taking hypertensive medications, and although there was no treatment effect on blood pressure, the DHA group had a significant decrease in heart rate at 24-weeks compared to baseline. There is debate as to whether nutritional supplementation can be beneficial alongside pharmaceutical treatments. The fact that over half of the participants in the current RCT were taking hypertension medication, and that those who took the DHA had a smaller increase in systolic blood pressure over the 12-month period, suggests that fish oil may provide additional benefit. Similarly, Freund-Levi et al. (2008) reported that 40% of participants in their trial were being treated with anti-depressant medication, and yet they found depression scores improved in non-ε4 carriers in the fish oil group.

The current RCT also found an APOE ε4 x treatment interaction with two of the well-being measures, the Geriatric Depression Scale (GDS) and the Geriatric Anxiety Inventory (GAI). Both GDS and GAI scores improved (decreased scores) in ε4 carriers in the DHA group,

whereas GDS and GAI scores increased in ε4 carriers in the placebo group. The result indicates that ε4 carriers may benefit from a DHA supplement with alleviating depression and anxiety symptoms. This may be partially explained by higher scores on the GDS and GAI in ε4 carriers at baseline. In contrast to this finding, the trial by Freund-Levi et al. (2008) found that in older adults with AD depression scores improved in non-ε4 carriers in the fish oil group, whereas ε4 carriers showed a positive treatment effect for agitation. These findings indicate that both non-ε4 carriers and ε4 carriers benefited from taking a fish oil supplement in terms of an improvement in well-being in AD. The link between genetics, and both depression and anxiety is well established (e.g., Sullivan, Neale, & Kendler, 2000). Depression and carrying the ε4 allele are both associated with higher levels of inflammation, and therefore it is possible that the fish oil was able to improve depression scores due to the interaction of these two factors. It is likely there is a mediating factor linking depression and the APOE ε4 allele, perhaps a personality trait, stress or dysfunction within the brain such as decreased activation of the frontal lobes (Carr & McNulty, 2016).

The results of the current RCT showed that there were no significant differences in the cognitive measures at baseline between ε4 and non-ε4 carriers, but that overall non-ε4 carriers improved more over the 12-month period than ε4 carriers in immediate memory, delayed memory, and total RBANS scores. However, this difference is not due to the treatment as the results showed no significant APOE ε4 x treatment effects for any of the cognitive measures. The lack of an APOE ε4 x treatment effect on cognition is in contrast to the trial by van de Rest et al. (2008a), which found ε4 carriers in both the low and high fish oil groups improved in an attention domain score, and the trial by Danthiir et al. (2018) where ε4 carriers improved significantly on several reasoning tasks. The total percentage of ε4 carriers in the current RCT was 30% which was very similar to another NZ based fish oil

RCT by Stonehouse et al. (2013), who found a total of 31% ε4 carriers in a sample of healthy adults (18 to 45 years of age). Both percentages are lower than those in the RCT by Quinn et al. (2010), where 57.7% of AD participants were found to be ε4 allele carriers. This is probably due to the increased risk for AD from carrying the ε4 allele. It is still unclear how the ε4 allele affects cognition with age, although the APOE ε2 x treatment effect on the RBANS total score indicates that carrying the ε2 allele may be a factor.

The current RCT was designed to replicate the trials by Sinn et al. (2012) and Lee et al. (2013). Sinn et al. found a significant improvement in scores on the GDS after 6-months, which the current RCT failed to replicate, although it did find a moderating effect from the ε4 allele. The participants in the trial by Sinn et al. showed higher scores at baseline than participants in the current RCT, which could partially explain the discrepancy. Lee et al. found that participants in the fish oil group improved significantly after 12-months of supplementation on several memory scores, but found no treatment effect on GDS. Both Sinn et al. and Lee et al. used the same cognitive measures at each testing time-point, which Lee et al. observed might have resulted in a practice effect, contributing to higher scores at the end of the trial. The current RCT tried to eliminate practice effects with the use of alternate forms of the RBANS, which could explain the discrepancy in this finding.

Lee et al. noted that participants from the trial were from a low socioeconomic background where the consumption of foods high in *n*-3 PUFAs was low due to financial constraints. Participants in Lee et al.'s trial began with DHA and EPA levels that were approximately half the baseline DHA and EPA levels of participants in the current RCT. This supports the idea that participants with lower levels at baseline may benefit more from taking a nutritional supplement. Levels of LA, EPA and DHA in the trial by Lee et al. were measured from plasma as a percentage of total plasma as opposed to red blood cells, and

this may also explain why mean baseline levels of LA in the fish oil group in trial by Lee et al. were lower than in the current RCT. This may also be a reflection of the Western-style diet of New Zealanders where consumption of *n*-6 PUFAs is higher than in Asian countries like Malaysia, where the trial by Lee et al. was based.

Lee et al. observed that participants were 'mildly impaired' but not 'in a more progressive stage', implying that the positive results were possibly due to the level of cognitive impairment. This raises the question about whether the participants in the current RCT were not sufficiently cognitively impaired, and therefore were less likely to benefit from a nutritional intervention. The participants had a higher than average pre-morbid functioning level, so it is also possible that a participant group with a lower pre-morbid functioning level may have benefited more from the fish oil supplement. The fact that mean IQ scores were 119, yet the baseline scores for the RBANS indexes were close to a quotient of 100 and mean TMT B percentile scores were below the 50th percentile, indicates that the group was cognitively impaired, but it is not clear whether a higher cognitive reserve prevented a treatment effect.

Although years of education was included as a covariate, education norms were not available, and therefore, not used to score the cognitive measures. This is a major limitation of the RBANS as a primary cognitive battery. The MMSE was used in the two trials by Rondanelli et al. (2012) and Lee et al. with scores of between 26 to 27 at baseline (considered to be 'normal' cognition), and both found a significant treatment effect. The supplement group in Rondanelli et al. improved by over one point on the MMSE and the placebo group decreased by over two points resulting in a significant effect, whereas in the trial by Lee et al. the fish oil group had improved by 0.2 of a point and the placebo group by 0.1 of a point on the MMSE. It is unclear what the pre-morbid functioning level or the IQ of

these participants were. Considering the variations in MCI criteria, it may be more useful to include a pre-morbid functioning task rather than a global scale such as the MMSE to gauge the baseline level of functioning of participants.

It is unclear whether the types of cognitive measures used in the current RCT contributed to the negative results. The current RCT used several similar cognitive measures as Sinn et al. and Lee et al. including Digit Span Forwards, Digit Span Backwards, Trail Making Test A and B and a version of the Stroop Test. One noteworthy difference from the current RCT was that Sinn et al. found a treatment effect for Initial Letter Fluency. In the current RCT Semantic Fluency was measured, and was combined with Picture Naming into an RBANS Language quotient score. Because reliability coefficients are higher for indexes than for subtests, the RBANS subtest scores are not usually reported; however, the analysis showed no differences between the two groups on scaled Semantic Fluency subtests scores at all-time points. Lee et al. found a significant effect using Digit Span Total, which was not replicated in the current RCT. There is support for the use of the RBANS as a tool for measuring cognitive change, particularly in older adults at risk for decline and with AD (e.g., Duff et al., 2005), although less so in amnestic MCI (Duff, Hobson, Beglinger, & O'Bryant, 2010). Participants in the current RCT were a combination of amnestic and non-amnestic MCI due to low numbers, so including only one MCI subgroup should be considered in future fish oil trials.

The inclusion criteria for the trial involved a dietary intake of fish and seafood below two servings per week, and self-reported fish consumption from calendar records indicated that participants consumed on average 1.14 servings per week. The significant positive correlations between baseline levels of DHA and the omega-3 index with baseline weekly fish consumption as measured by the nutritional screening questionnaire indicates that this

method of assessing fish consumption was reliable. The only difference between the DHA and placebo groups at baseline was in ARA levels, but both groups showed a similar decrease in ARA levels over the 12-month period. It is unclear why ARA levels decreased in the placebo group, especially since LA is the precursor to ARA and in the current RCT the placebo group was given 1857 mg of LA per day compared to less than 0.01 mg in the DHA group. Despite the high dose of LA in the current RCT, the placebo group showed a decrease in LA levels over the 12-month period, which suggests that LA may have been converted to fatty acids other than ARA. The influence of LA and ARA on cognition is not yet fully understood, although research shows that ARA levels tend to decrease with age, and that these levels are heavily influenced by EPA and DHA intake.

Nervonic acid levels increased by a similar amount in both groups, which could possibly explain the improvement found on the cognitive measures in both groups. Nervonic acid is an *n*-9 PUFA found in white matter, primarily in the myelin sheath of nerve fibres, and low levels have been implicated in attention-deficit hyperactivity disorder and psychosis (Chen Hsu, Hsu, Hwang, & Yang, 2004; Amminger et al., 2012). Nervonic acid is considered an important nutrient for neuronal growth and is typically found in maternal milk as well as in oily fish and seeds, although it can also be produced from oleic acid. The fish oil supplement in the current RCT contained nervonic acid (0.057 g/day), but it was only present in trace amounts in the placebo capsule (<0.01 g/day). The increase in nervonic acid levels in the placebo group could be due to an increased conversion rate from the oleic acid, as this was higher in the placebo capsule (0.866 g/day) than the DHA capsule (0.045 g/day).

9.2 The cross-sectional survey

The results of the cross-sectional survey showed that over 21% of NZ respondents used fish oil supplements, and that those who took fish oil supplements were more likely to regularly consume oily fish. It appears that fish oil is becoming a popular choice of nutritional supplementation, yet questions remain about current dietary recommendations as well as quality and sustainability issues. The current survey also found that only 6.8% of fish oil users stored their fish oil supplements in the refrigerator, and approximately 26% of fish oil users were taking a daily dose of fish oil that would meet the National Heart Foundation daily recommendations of 400 to 600 mg DHA and EPA. Dietary recommendations for ALA, EPA, DHA and total *n*-3 PUFAs and *n*-6 PUFAs have been very difficult to set, and consequently, recommendations for these nutrients vary widely around the world. Reasons for this difficulty include a lack of information about set dose-response relationships, uncertainty over conversion rates and the difficulty with differentiating the requirements of each fatty acid individually (Capra et al., 2006).

Since *n*-3 PUFAs and *n*-6 PUFAs compete for conversion enzymes, several dietary organisations decided to set *n*-3 PUFA:*n*-6 PUFA ratios of between 1:5 to 1:10, with a ratio over 1:10 indicating the need to consume more *n*-3 PUFAs. Some experts disagreed with this as a dietary recommendation, and instead suggested it may be helpful to set recommendations for plant and marine sources of *n*-3 PUFAs and *n*-6 PUFAs separately due to their different effects, and also because decreasing *n*-6 PUFAs does not produce the same effect as increasing *n*-3 PUFAs (de Deckere, Korver, Verschuren, & Katan, 1998). This led to the development of separate recommendations for LA, ALA, EPA and DHA based on the different physiological effects. Recommendations for PUFAs are often expressed as an adequate intake (AI), which is an estimate based on results from both observational studies

and RCTs. Currently in NZ the daily AI levels for women over 19 years of age are 8 g of LA, 800 mg of ALA and 90 mg of combined DHA + EPA; for men, 13 g of LA, 1300 mg of ALA and 160 mg of combined DHA + EPA. These equate to an *n*-3 PUFA to *n*-6 PUFA ratio of approximately 1:10. The suggested dietary target (SDT) or upper level at the 90th percentile is a level of intake that provides a 'potential benefit while still at a safe level'. In comparison to the AI, the SDT is set at 430 mg/day combined DHA + EPA for women and 610 mg/day for men, with an upper level of intake of combined EPA, DPA and DHA set at 3000 mg/day (Capra et al., 2006).

There is minimal information about the consumption of individual fatty acids by New Zealanders. The 2008/9 National Adult Nutrition Survey found that older adults between 51 and 70 years of age had a median intake of total PUFAs of 10.5 g/day, contrasted with 8.85 g/day for older adults over the age of 71, and a median intake of 11.35 g/day for all age groups combined. A review published in 2000 estimated that PUFA intake of adult New Zealanders was high, sitting at 15 g/day for LA, 1000 mg/day of ALA and slightly less than 200 mg/day of DHA + EPA (Eyres, 2000). Based on recommended AIs these figures are sufficient; however, they are not sufficient when based on SDTs. The question of whether the dietary intake of *n*-3 PUFAs and *n*-6 PUFAs in older adults in NZ is contributing to the increasing incidence of dementia is worthy of further research.

The ethics and sustainability of using fish to produce *n*-3 PUFA supplements is a very topical issue, especially in light of the high numbers of oxidised fish oil supplements. There has been a decline in wild marine fish stocks, partially due to the increased demand for fish and fishmeal due to population growth and rising disposable incomes. This is especially the case for fish high in *n*-3 PUFAs such as salmon, where the salmon available to consumers (and presumably used in supplements) is now primarily from farmed salmon (de Roos,

Sneddon, Sprague, Horgan, & Brouwer, 2017). A 2017 paper on the health properties of farmed fish found that the content of EPA and DHA has halved within farmed salmon in the last decade, which will no doubt hinder people from meeting their requirements from this food source (de Roos et al., 2017). Part of the reason for this decline is the type of food salmon are fed. In the wild, salmon typically eat other fish, squid, eels and plankton (all sources of EPA and DHA), whereas farmed salmon are typically fed soya or corn-based feed, lacking in *n*-3 PUFAs or even rapeseed oil, which contains ALA as opposed to EPA and DHA.

The source of *n*-3 PUFAs for fish oil supplements in RCTs is often unknown and varies among the trials. In the current RCT, the source of the *n*-3 PUFAs was sardines, a small short-lived wild fish, and therefore had no detectable levels of mercury. Due to declining fish stocks as well as the decline in *n*-3 PUFA content of certain types of fish, marine algae or seaweed is increasingly used as an alternative source of EPA and DHA. Algae oil supplements have been shown to significantly increase DHA levels and are also a source of other important nutrients including iodine and magnesium, and plant pigments such as chlorophyll. Two of the RCTs reviewed in Chapter 5 used an algae-based *n*-3 PUFA supplement – Quinn et al. (2010) and Vakhapova et al. (2010). The algae supplement used by Quinn et al. contained only DHA whereas the supplement used by Vakhapova et al. contained EPA, DHA and phosphatidylserine, a phospholipid found in plasma membranes that plays a crucial role in neuronal signal transduction. Quinn et al. found a lower decline in mean change in the ADAS-cog for non-ε4 carriers and Vakhapova et al. found an improvement in immediate recall. Quinn et al. found that plasma phospholipid levels and cerebrospinal fluid levels of DHA increased substantially from the algae supplement. Five participants who were screened for the current RCT were either vegetarian or vegan, and therefore, may have participated had the trial used an algae-based supplement.

The survey also contained a question aimed to see how willing people were to have a blood sample taken as part of a research study. The reason for including this question in the survey was due to the main supervisor's concerns that this would be a deterrent to participation. It turned out that 70% of respondents answered 'Yes' to this question, indicating that the majority of respondents would not be deterred from participating in a trial if a blood sample was required. Considering the importance of measuring RBC levels of fatty acids to analyse compliance and treatment effects as well as the need for APOE status, it was determined that having a blood sample taken would remain as a core criterion for the trial.

9.3 Implications

The results of the current RCT add to an already confusing picture about whether EPA and DHA from fish oil can help older adults with cognitive difficulties. Exactly why the results from these trials have been inconsistent is of significant interest, especially when epidemiological and nutritional-based research is relatively strong. Until now, it appeared that somewhere between cognitively healthy and dementia was where fish oil supplementation might be most beneficial. The fact that in the current RCT the only significant treatment effect detected was in systolic blood pressure may indicate that DHA works via homeostatic mechanisms, and therefore the EPA and DHA were used to correct a dysfunction within the cardiovascular system as opposed to within the neuronal system. The results show that overall, the mean cognitive scores were higher in the DHA group compared to the placebo group at the 12-month mark, but the difference did not reach statistical significance. Whether a longer supplement period (e.g., between 18 and 24 months) would result in a stronger treatment effect is unclear. Chew et al. (2015) used a 5-

year supplement period and Dangour et al. (2010) a 2-year period in cognitively healthy older adults, and yet neither study found a treatment effect. Whether these negative results were due to the lack of cognitive impairment is unclear, although Chew et al. found that lower cognitive function at baseline was associated with a lower education level, hypertension and stroke, highlighting the multifactorial nature of cognition in older adults.

The effects of supplementing with a nutrient can be very difficult to measure, especially in comparison to a substance such as a pharmaceutical agent, which typically targets a single receptor or type of tissue. Nutrients such as DHA and EPA as well as minerals and vitamins, can support multiple different functions within the body and exactly what the body does with these nutrients depends on several factors. These factors include current levels of the nutrient and its co-factors, current levels of disease and stress, and in the case of DHA, previous intake as well as current (and previous) intake of *n*-6 PUFAs and other *n*-3 PUFAs. This makes it very difficult to know what to measure (or control for) at a particular point in time without knowing a particular person's physiological and homeostatic state.

Another critical factor is that not all participants begin an RCT in the same physiological state. RCTs have typically measured demographic and cognitive states at baseline, but it is costly and time consuming to measure all components of a person's health. Efforts to measure baseline levels of DHA, EPA and other fatty acids in *n*-3 PUFA trials has made progress in this regard, but this type of analysis does not give any insight into exactly how the fatty acids will be used or stored in the body. Both EPA and DHA contribute significantly to the production of anti-inflammatory mediators, to a reduction in vaso-constriction, to thrombosis production within the vascular system, to protecting and regenerating various types of tissue, as well as playing an important role in vision and

energy production. Future trials may need to measure these effects to rule out reasons why DHA and EPA may not be contributing to an improvement in cognition.

The inconsistencies in the results from RCTs for other nutrients have been similarly problematic. For example, vitamin C has received considerable attention over the years, specifically in preventing the common cold, and yet vitamin C is involved in over eight major enzyme-dependent reactions within the body as an anti-oxidant agent, a lipid protector and in collagen, gastric juice and hormone production (Capra et al., 2006). Vitamin C also aids the absorption and stabilisation of other nutrients like iron, copper, vitamin A and folate. Therefore, how the body chooses to use the vitamin C when ingested depends on the current state of health within the body. For example, the turnover of vitamin C is markedly higher in smokers and consequently the RDA is higher in smokers (100 mg/day) than for non-smokers (between 30 to 60 mg/day) (Schectman, Byrd, & Hoffmann, 1991).

The trial by Witte et al. (2013) used MRI and found that in healthy older adults a fish oil supplement resulted in significant increases in fractional anisotropy, mean diffusivity and radial diffusivity, an indication of superior white matter structural integrity. The trial also found that the fish oil supplement increased grey matter regional volume in the left hippocampus, precuneus, superior temporal, inferior parietal and postcentral gyri, and right middle temporal gyrus. In contrast, Quinn et al. (2010) found no changes in total brain volume using MRI between two treatment groups of participants with AD. This suggests that fish oil supplementation may result in structural and functional changes within the neuronal system, but only at the pre-pathological stage. It is possible that a regular intake of *n*-3 PUFAs over the course of a lifetime could help to strengthen neuronal pathways from normal wear and tear, but not for severely damaged neuronal pathways. It is clear that genetic factors play a part in the physical and cognitive ageing process, although it is still

unclear as to whether EPA and DHA improve cognitive outcomes via genes, by targeting specific areas of the brain (and specific cognitive domains), or via broad-spectrum mechanisms such as reducing inflammation and oxidation (e.g., the decrease in inflammatory mediators in Yacong et al., 2017) and by improving synaptic transmission and cerebral flow – or all of the above. Understanding exactly how *n*-3 PUFAs, EPA and DHA work to improve cognition (or to prevent cognitive decline) needs to be explored further to understand who may benefit the most from taking a fish oil supplement.

The current survey found that over half of fish oil users take fish oils to 'improve brain functioning', and yet the results from the current RCT and the 23 RCTs reviewed in Chapter 5 suggest that the evidence for the use of fish oil supplementation in improving brain functioning in older adults (regardless of the level of impairment) is still not clear. Discrepancies between the evidence for fish consumption, the evidence for *n*-3 PUFA, EPA and DHA intake, the evidence for RBC and plasma *n*-3 PUFA levels, and results from RCTs suggests that the protective effect of fish consumption on cognition may be from more than just the *n*-3 PUFA content (Dacks et al., 2013). Fish contains other dietary and nutritional factors including vitamin D, calcium, zinc, selenium and protein, which in older adults have been shown to help with improving bone density, well-being, tissue strength, mobility and energy levels. The role and impact of culture and food habits on fish consumption is also an important factor (Gillette-Guyonnet et al., 2007). A RCT carried out in Japan used an *n*-3 PUFA enriched fish product in the form of a fish sausage as opposed to a capsulated product as the source of EPA and DHA (Hashimoto et al., 2012). The sausages contained a daily dose of 1720 mg of DHA and 407 mg of EPA and the trial found positive results in language copying from the MMSE and conflicting instructions from the Frontal Assessment Battery. The authors noted that compliance with consuming the fish sausages was very high due to

them being a traditional way of consuming fish as a form of snack for children in Japan. Fish is not only seen as a source of nutrients; foods from the sea (or from a river) play a very significant role in certain food cultures.

Fish is also typically part of an overall healthier diet, for example in the Mediterranean diet, and diets from Scandinavian and Northern Asian countries such as Japan. In contrast, the Western-style diet is typically simultaneously low in fish consumption and high in red meat, processed foods and *n*-6 PUFAs from fried foods and margarine, resulting in a lower ratio of *n*-3 PUFAs to *n*-6 PUFAs. The 2008/2009 New Zealand Adult Nutrition Survey showed that the primary food sources of protein of New Zealanders are from bread (11%), poultry (9%), milk (9%), beef and veal (8%), grains and pasta (7%), bread-based dishes (7%), fish and seafood (7%) and pork (5%), and that very little fat comes from fish and seafood (only 3.8%) compared to butter and margarine (9.3%) which are typically high in saturated fatty acids and *n*-6 PUFAs (New Zealand Adult Nutrition Survey, 2011). The National Adult Nutrition survey does not specify what types of fish and seafood New Zealanders eat. Fish and seafood are consumed less than other types of meat due to the smell and taste, to the high cost compared to other forms of protein, and because it spoils more quickly than other forms of protein like eggs and beef. Fish and seafood sources of *n*-3 PUFAs tend to be those that people like the least, including sardines, anchovies, tuna and herring, because of their strong or 'full-flavoured' taste. In addition to this, there are very few other food sources of *n*-3 PUFAs apart from fish and seafood in the Western diet.

The NZ diet, especially in older adults, is still based mainly on the old UK diet of 'meat and three vege'. When fish is consumed, it is typically in the form of 'fish and chips', which usually consists of a white fish (therefore with lower levels of *n*-3 PUFAs) along with potato chips cooked in vegetable oils that are high in *n*-6 PUFAs. However, in the last decade

or so, NZ's food culture has adopted several non-meat sources of *n*-3 PUFAs from other cultures as can be seen in the consumption of seaweed in sushi and chia seeds in certain cereals and bread. The traditional Māori diet on the other hand contains a significant number of foods from the sea or from rivers, a lot of them high in *n*-3 PUFAs such as eel, muttonbird, and shellfish such as paua and kina or sea urchin. It could be that the traditional Māori diet is not necessarily higher in fish and seafood in terms of quantity, but may include a wider range of types of seafood, and in particular, types that are high in *n*-3 PUFAs. Even though salmon is more likely to be part of a Western-style diet than a Polynesian diet, intensive farming means the PUFA content might be lower than in non-farmed *n*-3 PUFA foods like kina.

The current survey also found that fish oil supplement use was least likely in respondents who regularly eat nuts and seeds. Plant sources of *n*-3 PUFAs such as ALA are seen in most traditional diets around the world – linseeds in the Indian Ayurvedic tradition, leafy vegetables including *Perilla* from traditional Korean and Japanese food cultures, *Portulaca* (purslane) from both the Mediterranean and Central Europe, hemp seed from China, chia seeds from Central and South America, berries such as lingonberry from Nordic countries, gooseberry from China and rosehip berries from the indigenous people of North American as well as the Mayans. Both DHA and EPA can be synthesised from ALA, but due to limited conversion rates, most dietary organisations recommend eating fish and seafood to obtain sufficient amounts of DHA and EPA. However, there is still a need to promote the consumption of plant food sources of *n*-3 PUFAs as well, especially considering that the number of people opting for a vegetarian or vegan diet is increasing. Seaweed and algae hold promise, as certain strains of algae contain LA, ALA, EPA and DHA, providing a combination of all the necessary fatty acids in one food source. Algae are vegetarian

friendly, have a low carbon footprint, contain other important and often commonly deficient minerals such as iron, zinc and iodine alongside many other phytonutrients. The fatty acids in algae are stable, unlike the fatty acids in fish oil and are less likely to become oxidised (easier to manufacture and store), making them an attractive commercial product for capsulation. Like mussels, seaweed and algae can be farmed sustainably without causing detrimental effects to the environment.

As some nutritional experts have suggested, increasing foods high in *n*-3 PUFAs is more efficacious than decreasing foods high in *n*-6 PUFAs. This does not mean that efforts to reduce foods high in *n*-6 PUFAs are not worthwhile. Foods high in *n*-6 PUFAs that are likely targets are certain types of margarines, fried foods, processed biscuits and breads, pastries, crackers and cakes – all of which pose significant other concerns including the high sugar content and low overall nutritional content including fibre, and the high content of saturated and trans-fatty acids. These nutritional factors contribute to obesity, diabetes and other major chronic health conditions, all of which also increase the risk of cognitive decline and dementia. The idea of combining fish oil supplementation with other lifestyle interventions to reduce cognitive decline has led to the development of multi-domain interventional programs such as Multi-domain Alzheimer Preventive Trial, but so far with limited results (Chhetri et al., 2018). The best prevention approaches for cognitive decline in older adults are most likely to be multifactorial with multiple benefits; however, this design methodology poses a range of challenges for researchers.

9.4 Strengths and limitations of the trial

The main strength of the current RCT is that it was designed to replicate components of two published RCTs (Sinn et al., 2012; Lee et al., 2013) with a similar dose and similar

measures. Considering the ever-increasing number of fish oil RCTs (with widely varied design details and consequently varied results), it may be time to start replicating key design components to bring some order to the current confusion.

The current RCT had several limitations. The lack of educational norms for the RBANS was a major limitation, particularly given that the participant group was highly educated and showed high pre-morbid functioning. As a result, it was difficult to gauge the exact level of cognitive impairment of the participant group, which is important as it has been suggested that the negative results from trials could be due to participants not being impaired enough or having a high cognitive reserve. Participants in the trial were a mix of a community sample and those recruited from a memory clinic, whereas most other RCTs have been one or the other. At least four of the participants began the trial with pre-existing conditions (one reflux, two previous stroke, one bowel irregularities), which in hindsight should have excluded them from the trial, primarily for their safety and comfort. There is increasing evidence to suggest that a higher dose of EPA than used in the current RCT is required to see a noticeable change in depression scores, therefore future trials should consider using a supplement high in both DHA and EPA. The small sample size (and low power) restricted analysis, mainly concerning the APOE x treatment effects. The 12 participants who dropped out of the trial were not followed up, and therefore this data was missing. The decision was made not to perform an intention-to-treat analysis, as the small sample size with the high proportion of participant drop-outs would risk underestimating the efficacy of the treatment.

9.5 Conclusion

The current RCT aimed to investigate whether a 12-month course of a high DHA fish oil supplement could improve scores on cognitive and well-being measures in older adults with MCI. The trial found no evidence of a treatment effect on the cognitive measures, although a positive result was found for systolic blood pressure in favour of the DHA fish oil supplement. The trial also aimed to investigate the moderating effect of the APOE ε4 allele on the treatment, which was shown for scores on both the GDS and GAI in the DHA group. Despite the lack of treatment effect on the cognitive and well-being measures, the positive result for APOE ε4 carriers and on systolic blood pressure justifies further RCTs.

A poor diet underlies the main risk factors for dementia including obesity, diabetes, hypertension and depression, and is associated with the major health behaviours associated with cognitive decline, including physical inactivity, smoking and low educational attainment. A recent meta-analysis of 15 cohort studies involving over one million participants found that a DASH-like diet (low in salt and high in vegetables, fruit, nuts and legumes) significantly reduces the risk for cardiovascular disease, cancer and type 2 diabetes, but not neurodegenerative diseases such as dementia (Schwingshackl & Hoffmann, 2015). Therefore, it could be that diet may provide a beneficial effect on cognitive decline via effects on the cardiovascular, digestive and endocrine systems. It could be that measuring the cumulative effects of diet on cognitive decline independently of these factors is difficult to do with a RCT methodology. The inconsistencies from RCTs is of concern, especially for consumers who amid conflicting media reports, try to decide whether taking a fish oil supplement or purchasing salmon is worth the time and expense. It is very difficult to study the effects of EPA and DHA without the influence of other metabolic

factors, for example, those that control LA and ALA intake and metabolism, and without being able to control and measure what the body does with the DHA and EPA.

There is no doubt that *n*-3 PUFAs play an important role in the neuronal system as well as in the homeostatic mechanisms used to control inflammation, vasodilation and tissue regeneration. There is also no question about how crucial DHA is to the development of the nervous system, but it is still unclear exactly what role it plays within the neuronal system in the later years. Epidemiological research indicates that fish consumption is part of a diet that protects older adults from a whole range of age-related chronic diseases, but the exact role it plays in reducing or preventing severe forms of cognitive decline is still unclear. Diminishing fish stocks highlight the need to promote plant sources of *n*-3 PUFAs, in particular marine algae, which would ensure a sufficient intake of EPA and DHA alongside other important nutrients.

Efforts by researchers to test the efficacy of EPA and DHA by way of RCTs has resulted in painting a very confusing picture. Trying to decipher the details of these RCTs has researchers questioning the impact of dose, duration and measures, together with baseline demographic factors like lifestyle and health status. Whether any of these factors can explain the inconsistencies in the results is yet to be determined. What remains is evidence that suggests that what we eat is an important part of the dementia puzzle, alongside the quality of our social connections, our educational attainment, the amount of physical activity we do, and our general health, in particular the health of the vascular and cardiovascular systems as well as our mental health. We must of course consider the cohort and generational nature of the older adults we have tested and collected data on so far. The new generations are faced with a new world, with different eating patterns, different forms of physical exercise, and different ways of socially interacting with others.

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Appendices

Appendix A

Statement of contribution

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Alexia Mengelberg

Name/Title of Principal Supervisor: Professor Janet Leathem

Name of Published Research Output and full reference:
Mengelberg, A., Leathem, J., & Podd, J. (In 2018 print). Fish oil supplementation use in New Zealand. Complementary Therapies in Clinical Practice, 33, 118-123.

In which Chapter is the Published Work: 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 85%

~~and / or~~

- ~~Describe the contribution that the candidate has made to the Published Work:~~

Candidate's Signature — 5/09/2018 Date

Principal Supervisor's signature — 6/09/2018 Date

Appendix B. Survey advertisement

Would you like to win a $100 supermarket voucher?

Complete an online survey and you can go into a draw to win a $100 supermarket voucher.

What is the survey about? It is part of a PhD project which is investigating the types of nutritional supplements people take as well as their dietary and lifestyle habits

Who can complete the survey? Anyone over 18 years of age

How? Use the following link - https://jfe.qualtrics.com/form/SV_7WgN1Cs2E3fUWYl

MASSEY UNIVERSITY | School of PSYCHOLOGY

Researcher/PhD student:
Alexia Mengelberg
School of Psychology
Massey University Wellington
[redacted]@gmail.com
(04) [redacted]

This project has been reviewed and approved by the Central Health and Disability Ethics Committee. Ethics Reference: 14/CEN/148/AMO1

Supplement Survey https://jfe.qualtrics.com/form/SV_7WgN1Cs2E3fUWYl

Appendix C. Supplement survey

Section 1 – Consent

Thank you for participating in this questionnaire. Your participation implies consent. You have the right to decline to answer any particular question.

I have read and understood the information sheet for this study and consent to collection of my responses.

- Yes
- No

Section 2 – Demographics

1. What is your current age?

- 18-29 years old
- 30-39 years old
- 40-49 years old
- 50-64 years old
- 65-85 years old
- 86 years and older

2. What is your gender?

- Male
- Female
- Other

3. Which town/city do you live in?

- Whangarei
- Auckland
- Hamilton
- Tauranga
- Taupo
- Napier-Hastings
- Gisborne
- Palmerston North
- New Plymouth
- Whanganui/Wanganui
- Wellington
- Nelson
- Christchurch
- Dunedin
- Invercargill

4. Which ethnic groups do you belong to?

- New Zealand European/Pakeha
- New Zealand Māori
- Pacific Islander
- Chinese
- Indian
- Other (please specify)

5. What is the highest qualification you have achieved?

- High school attendance
- School certificate or NCEA Level 1
- University Entrance, Bursary or NCEA level 3
- Tertiary certificate or diploma (not a degree)
- Bachelor's degree – For example: BA, BSc
- Postgraduate – For example: Honours, Masters, PhD or postgraduate diploma

6. What is your annual salary?

- 0 – $14,999 per year
- $15,000 – $24,999 per year
- $25,000 – $44,999 per year
- $45,000 – $64,999 per year
- $65,000 – $84,999 per year
- $85,000 – $104,999 per year
- $105,000 – $149,999 per year
- $150,000 + per year

7. Are you currently taking any medications prescribed by a GP/Medical doctor/Physician?

- o Yes
- o No

8. How would you rate your health status?

- o Excellent
- o Good
- o Fair
- o Poor

9. What is your current height?
Please use either metric or imperial measures (eg. 183cm or 6'3")
Open

10. What is your current weight?
Please use either metric or imperial measures (eg. 70kg or 154 pounds or 11 stones 3lbs)
Open

11. Do you smoke tobacco cigarettes?

- o Yes
- o No

12. Would you be willing to have a blood sample taken if you were part of a research study?

- o Yes
- o No

Section 3 – Fish oils

1. Do you take fish oil supplements?

- o Yes
- o No

2. How often do you take fish oil supplements?

- o Every day
- o 4-6 times per week
- o 1-3 times per week
- o Several times a month
- o About once a month
- o Less than once a month

3. How long have you been taking fish oil supplements?

- o Less than 3 months
- o Between 3 to 6 months
- o Between 6 to 12 months
- o More than 12 months

4. Why did you first start taking fish oil supplements?

- o General well-being
- o To improve brain functioning
- o For joint pain and/or inflammation
- o To lower cholesterol levels
- o Dietary insufficiency
- o Other
- o Recommended to take them by a health professional e.g. GP, nutritionist, naturopath
- o Heard about them on the radio/TV
- o Read about them in a magazine
- o Read about them on the internet
- o Was told about them by a friend or family

5. Have you ever taken a break from taking fish oil supplements for more than 1 month?

- o Yes — Why? Open
- o No

6. What brand of fish oil supplements are you currently taking? Open

7. What dose of fish oil supplements are you currently taking?
e.g. 2 capsules containing 300 mg of DHA and 120 mg of EPA
Open

8. Where do you store your fish oil supplements?

- o On my bench top
- o In the pantry/food cupboard
- o In the refrigerator
- o Other

9. Have you noticed any good or bad health effects from taking fish oil supplements?

- o Yes
- o No

10. Do you feel you know enough about fish oil products and their use?

- o Yes
- o No

Section 4 – Supplement use

1. Do you take any other types of supplements?

- o Multi-vitamins and minerals
- o Single vitamins e.g. Vitamin C
- o Single minerals e.g. Calcium, Magnesium, Iron
- o Probiotics e.g., Acidophilus
- o Herbal remedies e.g., Ginkgo, Echinacea, Ginseng, St. Johns Wort
- o Co-Q10
- o Glucosamine/Chondroitin
- o Other
- o I don't take any supplements

2. How often do you take these supplements?

- o Every day
- o 4-6 times per week
- o 1-3 times per week
- o Several times a month
- o About once a month
- o Less than once a month

3. Why do you take these supplements?

- o For a particular medical condition
- o General well-being
- o Dietary insufficiency
- o Recommended to take them by a health professional e.g. GP, nutritionist, naturopath
- o Heard about them on the radio/TV
- o Read about them in a magazine
- o Read about them on the internet
- o Was told about them by a friend or family member
- o Other

4. Where do you purchase your supplements? *Tick all that apply*

- o Pharmacy
- o Specialty health store e.g. Health 2000
- o Supermarket
- o Online
- o Phone/Mail order
- o Other

5. What influences your decision when selecting your supplements?

- o Brand
- o On sale/special price
- o Availability
- o Advertising claims

- Other

6. Have you ever told your GP/Medical doctor/Physician that you take supplementation?

- Yes
- No Why not? Open

Section 5 – Your diet and exercise habits

1. How often do you eat oily fish such as salmon, tuna, mackerel, anchovies including tinned tuna, eel or sardines?

- Never
- Rarely
- Once a month
- Once a fortnight
- Once a week
- 1-2 times per week
- More than 2 times per week

2. How often do you eat white fish or seafood including shellfish and crustaceans such as prawns/shrimps?
White fish includes snapper, terakihi, moki, john dory, hoki, gropper, monkfish, cod, bluenose, gurnard, sole

- Never
- Rarely
- Once a month
- Once a fortnight
- Once a week
- 1-2 times per week
- More than 2 times per week

3. How often do you eat nuts and seeds such as walnuts, almonds, sunflower seeds, pumpkin seeds, flaxseeds or oils such as flaxseed oil or hempseed oil?

- Never
- Rarely
- Once a month
- Once a fortnight
- Once a week
- 1-2 times per week
- More than 2 times per week

4. How often do you eat free range eggs, free range chicken or grass fed meat (e.g., beef, lamb, vension)?

- Never
- Rarely
- Once a month
- Once a week
- 1-2 times per week
- More than 2 times per week

5. How many portions of fresh fruits and vegetables do you eat per day? One portion is equal to one handful

- On average, less than 1 portion per day
- About 1 portion per day
- 2-3 portions per day
- 4-5 portions per day
- 6-7 portions per day
- 8+ portions per day

6. How often do you engage in exercise for longer than 30 minutes at a time?

- Never
- Rarely
- Once a month
- Once a week
- 1-2 times per week
- 3-4 times per week
- 5-6 times per week

Appendix D. Screening questionnaire

Participant Screening questionnaire **Assigned ID number** _____

Name _____ **Date of phone call** _____

Stated would be willing to be part of the study? **No** ***/ Yes*** **Why not?** _____

Do you fit between the age of 60 and 90? Y / N **How old are you?** _____

Have you taken any fish oil capsules over the last 6 months? Yes / No _____

Are you willing to not take a fish oil supplement of your own during the trial? Yes / No

Do you eat 2 or more servings of oily fish per week? Yes / No

Do you have any allergies to any type of fish or seafood? Yes / No

So are you willing to have two blood samples taken? Yes / No

Any major medical conditions including head injury, recent or expected major surgery?

Yes / No _____

Do you take any medications? _____

Are you planning on being away for any period of time during the next 15 months? Yes /No

Do you have any problems swallowing soft-gel capsules? Yes / No

Can you attend four testing sessions at the psychology clinic at Massey University? Yes / No

Are you fluent in English? Yes / No

Have you or your friends and family noticed a change in your memory or cognition lately?

Do you live independently of a carer or home help? Y / N

Fits the inclusion criteria Yes / No

Great, you fit the project criteria, would you be willing to participate in the trial? Y / N

Address _____

_____

Sent Information sheet and Consent form ☐

2 week call ☐

Arrange first testing session date and time

_____

Appendix E. Trial leaflet and newspaper advertisement

Would you like to be involved in a PhD research project looking at ageing and memory?

What is the project about? It is part of a clinical trial to see whether taking a fish oil supplement can improve memory and thinking abilities in older adults

Who can participate in the project? Anyone over 60 years of age who thinks they may have a mild memory problem

How do I found out more? Call (04) or email @gmail.com

MASSEY UNIVERSITY | School of PSYCHOLOGY

Researcher/PhD student: Alexia Mengelberg
School of Psychology, Massey University Wellington

This project has been reviewed and approved by the Central Health and Disability Ethics Committee (HDEC) Ethics reference: 14/CEN/148/AM02

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Would you like to be involved in a PhD research project looking at ageing and memory?

What is the project about? it is part of a clinical trial to see whether taking a fish oil supplement can improve memory and thinking abilities in older adults.

Who can participate in the project? Anyone over 60 years of age who thinks they may have a mild memory problem.

How do I find out more? Contact the lead researcher Alexia Mengelberg on (04) or email at @gmail.com

Appendix F. Participant information sheet and consent form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Participant Information Sheet

Study Title: Investigating the effects of fish oil supplements on cognitive performance
Locality: Psychology Clinic at Massey University of Wellington
Address: 24 King Street, Mount Cook (near the Basin Reserve)
Lead researcher: Alexia Mengelberg
Contact phone number: (04) or (021)
Ethics Committee reference number: 14/CEN/148/AMO1

You are invited to take part in a study on omega-3 fish oil supplements and cognition. Whether or not you take part is your choice. If you do not want to take part, you do not have to give a reason, and it will not affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This document is 6 pages long, including a participant information sheet and a consent form. The participant information sheet will help you decide if you would like to take part in the study. It explains why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

Please make sure you have read and understood all the pages. You will receive a phone call once you have had time to read the information. This will give you a chance to ask any questions you might have about the study. If you agree to take part in this study, you will be asked to sign the consent form and return it using the self-addressed envelope enclosed. You can keep the participant information sheet. You will receive a copy of the signed consent form at the first testing session.

WHAT IS THE PURPOSE OF THE STUDY?

The aim of this study is to look at whether omega-3 fish oil supplements can improve thinking and reasoning abilities in older adults between the age of 60 and 90. The research concerning omega-3 fish oil supplementation for improving memory is very positive. It is hoped that the current study will contribute to a clearer understanding of the benefits of omega-3 fish oils for the prevention of conditions such as depression and dementia.

You have been chosen to participate in this study because you have a mild memory problem and it seems you might benefit from taking an omega-3 fish oil supplement. It is important that you do not have an allergy to seafood or have any difficulty swallowing soft-gel capsules. Please talk the lead researcher if you have any further questions about this.

This study will be placebo-controlled, double-blind and randomised. 'Placebo-controlled' means that half of the participants in this study will be given fish oil capsules and half of the participants will be given placebo capsules containing sunflower oil. 'Double-blind' means that neither you nor the lead researcher will know which capsules you will be given until the end of the study. This double-blind procedure ensures that we do not bias the results. 'Randomised' is ensures that there is an equal chance that you will be given the fish oil capsules or the placebo capsules.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

This study will begin in December 2015 and will finish in March 2017.

During the study there will be four testing sessions. All testing sessions will take place at the Psychology clinic at Massey University, 24 King Street, Mount Cook (near the Basin Reserve). It is important that you attend all four testing sessions.

The first testing session will take place sometime between December 2015 and February 2016. This will be called the baseline testing session and will take place before you start taking the capsules. The baseline testing session will take 2 hours to complete. It will involve 15 minutes to collect demographic information, 60 minutes of psychological tests then a 15 minute refreshment break, followed by 30 minutes of questions about your diet and general well-being.

You will be required to take the capsules for a 12-month period from the 1st of March 2016 until the 28th of February 2017. During these 12 months you will need to take 3 capsules per day after breakfast with water. Please store the capsules in the fridge. You will be given your first 4 bottles of supplements at the end of the baseline testing session. At the end of each month, I will contact you via telephone to answer any questions you may have about taking the capsules or about the study. You will also be asked to record on a monthly calendar your daily consumption of fish and seafood.

You will be required to have two lots of blood samples taken during the course of this study. The first sample will be after the baseline testing session and the second sample will be after the fourth testing session at the end of the study. You will need to go to the outpatients area at Wellington Regional Hospital on Riddiford Street in Newtown to have the blood samples taken. Please find enclosed a map of both the outpatients area at the Wellington Regional Hospital as well as the Psychology Clinic at Massey University. You will be given further information about the blood testing part of this study at the baseline testing session.

The second testing session will take place in September 2016. This second testing session will take 1 and ¼ hours to complete and will include 45 minutes of psychological tests and 30 minutes of questions about your well-being. Please remember to return your finished bottles of capsules at this session time along with any unused capsules.

The third testing session will take place in December 2016. This third testing session will take 1 and ¼ hours to complete. It will include 45 minutes of psychological tests and 30 minutes of questions about your well-being. Please remember to return your bottle of capsules at this session time along with any unused capsules.

The fourth testing session will take place in March 2017. This follow-up testing session will take 1 and ½ hours to complete. It will involve 45 minutes of psychological tests, followed by 45 minutes of questions about your diet and general well-being. Once the fourth testing session is over your participation in this study is complete. Again, please remember to return your bottle of capsules at this session time along with any unused capsules. Please return the calendar at this session too.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

Clinical research has shown that omega-3 fish oils can improve your ability to remember information faster. You may experience this effect during the trial or you may experience no effect at all.

The potential adverse effects or side effects from the capsules are minimal. If you experience any discomfort from swallowing the capsules or digestive problems such as nausea of reflux after taking the capsules please contact the lead researcher as soon as possible.

This study has been approved by the Central Health and Disability Ethics Committee and has been funded by the Massey University Postgraduate Research Fund, the Oakley Mental Health Foundation, a small project grant from the Neurological Foundation and the Hope-Selwyn Foundation Scholarship in Ageing.

WHAT IS A GENETIC TEST?

As part of this study, we will test to see if you carry a particular genetic allele in your blood. You can choose whether or not you would like to be told the results of this test**.**

We are all different in terms of our genetic make-up. This study is interested in a particular gene called the APOE4 allele. Research has also shown that people who have the APOE4 allele are more likely to benefit from a diet rich in omega-3 fish oils. Research has also shown that people who have the APOE4 allele are slightly more likely to develop Alzheimer's disease later in life than those who do not.

If you do carry the APOE4 allele this may have implications for your life or health insurance and for your family, as there is a small chance that it may have been passed onto your children and grandchildren. Please ask the lead researcher if you have any further questions about this.

WHO PAYS FOR THE STUDY?

You will be reimbursed for participating in this study with a $20 supermarket voucher for each testing session you attend. You will not be expected to pay any money to participate in this study.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, which is unlikely, you may be eligible for compensation from ACC just as you would be if you were injured in an accident at work or at home. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study will not affect your cover.

WHAT ARE MY RIGHTS?

Your participation in this study is entirely voluntary. You are free to decline to participate. If you do agree to participate in this study, you may withdraw at any time without being penalized for doing so. You have the right to access information collected about you as part of the study. Please contact the lead researcher directly if you would like to do so.

You will be informed of any new information that becomes available during the study during the monthly phone calls.

WHAT HAPPENS AFTER THE STUDY?

You can choose to be sent an information sheet with the results of the study. These results will be summarized and will be in the form of grouped test results. No individual results will be identifiable.

Research findings will not be associated with the names of the participants, and all records will be stored securely and then destroyed after ten years. Blood samples will be destroyed once they have been analysed.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact the lead researcher:

Alexia Mengelberg, PhD student
(04) or (021)
@gmail.com

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@hdc.org.nz

You can also contact the Central Health and Disability Ethics Committee (HDEC) that approved this study on:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz

For Māori health support, please contact Te Roopu Awhina:
Phone:
Email:

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Consent Form

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	Yes ☐	No ☐
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	No ☐
I have had the opportunity to use a legal representative, whānau / family support or a friend to help me ask questions and understand the study.	Yes ☐	No ☐
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes ☐	No ☐
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes ☐	No ☐
I consent to the research staff collecting and processing my information, including information about my health.	Yes ☐	No ☐
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	No ☐
I agree to my blood samples being disposed of using established guidelines for discarding biohazard waste.	Yes ☐	No ☐
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethic Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes ☐	No ☐
I understand the compensation provisions in case of injury during the study.	Yes ☐	No ☐
I know who to contact if I have any questions about the study in general.	Yes ☐	No ☐
I understand my responsibilities as a study participant.	Yes ☐	No ☐

I wish to receive the results of the genetic testing.	Yes ☐	No ☐
I wish to receive a summary of the results from the study.	Yes ☐	No ☐

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

Appendix G. Calendar example page

March 2017

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
		1 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	2 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	3 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	4 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	5 Taken pills Y/N Ate fish Y/N Felt unwell Y/N
6 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	7 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	8 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	9 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	10 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	11 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	12 Taken pills Y/N Ate fish Y/N Felt unwell Y/N
12 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	14 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	15 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	16 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	17 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	18 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	19 Taken pills Y/N Ate fish Y/N Felt unwell Y/N
20 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	21 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	22 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	23 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	24 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	25 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	26 Taken pills Y/N Ate fish Y/N Felt unwell Y/N
27 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	28 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	29 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	30 Taken pills Y/N Ate fish Y/N Felt unwell Y/N	31 Taken pills Y/N Ate fish Y/N Felt unwell Y/N		

Appendix H. Patient form

Clinical Trials and Studies

Fish Oil Supplementation Study (FOSS)

Subject Number:	Principal Investigator: Alexia Mengelberg
Patient Initials:	Contact: Alexia Mengelberg School of Psychology Massey University PO Box 756 Wellington 6140
Sex:	
Date of Birth: / /	E: T: M:

Tubes Required:
Visit 2
2 x 5mL EDTA or Heparin Lithium Green

Performed at CCDHB Laboratory
☐ FOSS Visit 2
☐ APO – E genotyping (send to CHL)

Laboratory Use Only	
Data Entry Instructions:	Temporary number (Last name: Subject # / First Name: Patient Initials)
Doctor Code:	ZFOSS
Location Code:	ZFOSS
Specimen Type:	EDTA or Heparin Green
Test Set:	ZNR, SNB (Specimen Note: Specimen forwarded to Clinical trials for processing"
Reference:	FOSS
Processing Instructions:	*Give form & EDTA tube to Trails and Studies.*

Date Specimen Taken: ___/___/___

Time Specimen Taken: ____:_____

Taken By:_____

Appendix I. Ethics approval

Health and Disability Ethics Committees

Health and Disability Ethics Committees
1 The Terrace
C/- MEDSAFE, Level 6, Deloitte House
10 Brandon Street
PO Box 5013
Wellington
6011

hdecs@moh.govt.nz

28 October 2014

Miss Alexia Mengelberg
Postgraduate Suite 7CO7
School of Psychology
Massey University
PO Box 756
Wellington 6140

Dear Miss Mengelberg

Re:	Ethics ref:	14/CEN/148
	Study title:	Investigating the effects of omega-3 fatty acid supplementation on cognitive performance in older adults with mild cognitive impairment.

I am pleased to advise that this application has been *approved* by the Central Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Central Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
2. Before the study commences at *any* locality in New Zealand, it must be registered in a WHO-approved clinical trials registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au).
3. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

Appendix J. Nutritional Screening Questionnaire

Name _____ Date _____ ID _____

Dietary questionnaire

a. Do you consume any type of fish or seafood?

Yes □ No □

If yes, on average, how many servings of fish and seafood do you consume per ***week*****?**

Consider all types e.g. fresh, frozen or tinned? (*Please **mark one** only*)

(A 'serving' = 100 – 120 g of fish or seafood (the size of an adults hand palm)
E.g. 1 small tin of fish = 1 serving per week

Per Week

□ None □ < 1 serving □ 1-3 servings
□ 4-6 servings

b. How often do you consume the following? (Please tick all categories)

Fresh/Frozen/Canned/Smoked Fish								
Please circle the types of fish normally consumed:	**Never**	**< 1x/ month**	**1-3x/ month**	**1x/ week**	**2-4x/ week**	**5-6x/ week**	**1x/ day**	**2+x/ day**
Fish fried in batter (from fish & chips shop)								
Albacore tuna, salmon, sardines, warehou, herring, kahawai, swordfish, carp, dogfish, gemfish, Alfonsino, rudderfish, anchovies								
Mackerel, snapper, oreo, barracouta, trevally, dory, trout, eel								
Tuna (canned), hoki, dory, gurnard, hake, kingfish, cod, tarakihi, groper, flounder								

Fresh/Frozen/Canned/Smoked Fish								
Please circle the types of fish normally consumed:	**Never**	**< 1x/ month**	**1-3x/ month**	**1x/ week**	**2-4x/ week**	**5-6x/ week**	**1x/ day**	**2+x/ day**
Crumbed fish Patties /Cakes / Fingers/ Nuggets/ Portions								
Green mussels, Squid								
Shellfish: Cockles, kina, oysters, paua, scallops, shrimp/prawn, pipi, roe								
Other (*please specify any other fish which is normally eaten but not mentioned above*)								
Omega 3 enriched products								
Omega 3 enriched milk or soy milk e.g. Vital or Essential								
Omega 3 enriched bread e.g. Goodness Omega 3								
Other (*please specify any other omega-3 enriched products that you normally eat but is not mentioned above*)								

Appendix K. Example trial individual cognitive report

Date
Address

Dear

Please find below a summary of the individual results of the cognitive screening tests that you underwent as part of the fish oil supplement and cognition trial. As you are aware the tests were conducted for a specific research purpose and so not what would normally be conducted for a clinical purpose.

The tests administered and your results are shown below:

Testing session (TS)		**1st TS**	**2nd TS**	**3rd TS**	**4th TS**
Test Administered	*Measures*	*Quotient*[1]			
Repeatable Battery of Neuropsychological Status (RBANS)	Immediate memory	109	85	94	94
	Visuospatial/Construction	100	72	72	75
	Language	99	88	92	92
	Attention	132	112	128	115
	Delayed memory	107	75	98	71
	TOTAL	113	82	95	85
Coin rotation task	Fine motor speed	88	71	79	65
Stroop colour-word test	Divided attention	116	113	102	114
Digit span backwards	Working memory	113	108	113	108
Trial making test B	Mental flexibility	125	70	124	113

1 Average is 100, average range is between 90-110. Scores between 80-89 are low average, 70-79 are well below average. Scores between 110-119 are high average.

Test Administered	*Measures*	*Score*[2,3]			
Geriatric Depression Scale GDS	Depression	1	1	1	2
Geriatric Anxiety Inventory GAI	Anxiety	0	0	0	0

2 GDS score – 0-4=No indication of depression, 5-9=Some indication of depression, >10=Indication of depression

3 GAI score – 0-8=Absence of anxiety, >9=Presence of anxiety

Test Administered	*Measures*	*Score*[4]			
SF-36 Physical	Self-reported physical health	44.07	56.76	45.74	41.7
SF-36 Mental	Self-reported mental health	61.46	53	60.63	64.45

4 Average is 50, average range is generally between 40-60

The grouped results which will show any differences between the fish oil and placebo groups will also be forwarded to you. If you have any queries about these test results, please contact me directly on (04) or via email: @gmail.com

Thank you again for your participation in the trial.

Kind regards,
Alexia Mengelberg
PhD candidate and lead researcher
School of Psychology, PO Box 756, Massey University
Wellington 6014

Appendix L. Trial grouped summary report

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date
Dear

Please find below a summary of the grouped results from the omega-3 fish oil trial that you were part of within the School of Psychology at Massey University in Wellington.

I want to thank you again for your contribution to the trial. The trial was a great success, and this is largely due to your time and effort.

The aim of the omega-3 fish oil trial was to answer two main questions:

1. Can an omega-3 fish oil supplement improve memory and cognition in older adults?
2. How does the APOE ε4 allele affect cognition and well-being in older adults?

The results of the trial found that the fish oil supplement had no effect on memory and cognition. This result is based on the finding that there were no differences between the fish oil group and the placebo group on the scores of the cognitive tests.

The trial found that the fish oil supplement had a significant effect on systolic blood pressure. Systolic blood pressure is the 'top' number of a blood pressure reading e.g., the number 140 of a blood pressure reading of 140/80. Systolic blood pressure refers to the amount of pressure within the arteries while the heart is contracting, and an increase in blood pressure is related to an increase in artery stiffness, which is common with age. The trial found that systolic blood pressure increased by 1.83 mmHg units in the fish oil group compared to a much bigger increase of 10.7 mmHg units in the placebo group. This finding indicates that the fish oil supplement resulted in a smaller increase in systolic blood pressure over the 12-month supplement period.

The trial also found that depression and anxiety scores were improved in APOE ε4 carriers from taking a fish oil supplement. We were interested in the APOE gene because previous research has shown that people who carry the APOE ε4 allele have an increased risk of developing Alzheimer's disease, and also that people with the APOE ε4 allele may benefit from taking a fish oil supplement. The results from the trial show that APOE ε4 carriers may benefit from taking a fish oil supplement because it can alleviate symptoms associated with depression and anxiety.

Te Kunenga ki Pūrehuroa
School of Psychology
PO Box 756, Wellington 6140, New Zealand **T** +64 4 8015799 **www.massey.ac.nz**

The results of this trial will make a significant contribution to our understanding of how omega-3 fatty acids and fish oil supplements can help improve health and well-being in older adults. Due to the success of the trial, the results will be complied into a journal manuscript and submitted to an international journal for publication. The results in the paper will be grouped results, so no individual results will be identifiable.

Lastly, I would like to apologise for the delay in getting these grouped results to you – I really do appreciate your patience. If you have any queries about the trial or these results, please contact me on (021) or via email at @gmail.com

Kind regards,

Alexia Mengelberg
PhD candidate and lead researcher
School of Psychology
PO Box 756
Massey University
Wellington 6014

Appendix M. APOE fact sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

APOE genetic test – Fact sheet

Here are some important facts to know about the APOE genetic test

What is APOE?

- APOE is the short name for a protein called Apolipoprotein E which is found in the liver and brain
- APOE's main role is to process and carry cholesterol and fats around the body.
- There are 3 alleles or types of the APOE protein – APOE2, APOE3 and APOE4. Each one has a slightly different role in the body.
- We each have two of the three types of APOE – we get one from each of our parents. We can either have two of the same type e.g., APOE3 and APOE3 or two which are different e.g., APOE3 and APOE4.
- We can test which two types of the APOE protein you have from a blood sample.
- If someone has APOE3 and APOE4, the results of the test will look like this – Apolipoprotein E genotype: E3/4
- Approximately 10% of people have at least one APOE2, 75% have APOE3 and 15% have APOE4.

Why did we test for the APOE protein?

- Research has shown that people who have one or two APOE4 types have a small increased risk of developing Alzheimer's disease and also that people with the APOE4 type can benefit from taking a fish oil supplement.

What does this mean for me?

- It is important to remember that having an APOE4 type does not mean you will develop Alzheimer's disease; it simply means you have a slightly higher chance than if you didn't have the APOE4 type. There are many other factors which will affect your chance of developing dementia such as your diet, how much exercise you do, your cardiovascular health and how often you engage in mental or brain activities such as word puzzles, playing a musical instrument or reading.

Want to know more information?

- Try the internet – http://en.wikipedia.org/wiki/Apolipoprotein_E
- Or ask your nurse or GP or a registered genetic counsellor

Study Title: Investigating the effects of fish oil supplements on cognitive performance
Lead researcher: Alexia Mengelberg **Email:** @gmail.com
Contact phone number: (04) or (021)

Te Kunenga ki Pūrehuroa
School of Psychology
PO Box 756, Wellington 6140, New Zealand **T** +64 4 8015799 www.massey.ac.nz

Appendix N. Data from baseline, 6- and 12-months

Table N1. Data for cognitive measures at baseline and at 6- and 12-month follow-up

Treatment	Baseline [1]	6 months	12 months
	RBANS Total quotient score		
DHA	101.90 (11.87)	104.00 (11.90)	108.40 (12.77)
Placebo	100.30 (11.89)	104.73 (11.47)	105.47 (11.20)
	RBANS Immediate Memory quotient score		
DHA	104.83 (16.19)	108.57 (16.88)	110.03 (13.59)
Placebo	105.33 (13.89)	109.43 (10.71)	109.87 (13.79)
	RBANS Visuospatial/Constructional quotient score		
DHA	96.63 (9.66)	95.73 (8.96)	98.37 (8.85)
Placebo	92.87 (12.67)	94.63 (9.80)	91.43 (11.79)
	RBANS Language quotient score		
DHA	103.97 (13.76)	100.40 (10.24)	107.03 (10.07)
Placebo	103.17 (12.79)	104.17 (8.15)	106.70 (7.76)
	RBANS Attention quotient score		
DHA	103.67 (13.79)	105.20 (13.79)	110.37 (15.24)
Placebo	102.90 (13.32)	106.63 (15.59)	109.80 (13.85)
	RBANS Delayed Memory quotient score		
DHA	98.20 (11.26)	105.47 (10.28)	104.50 (11.95)
Placebo	98.33 (12.66)	103.13 (13.71)	102.87 (12.87)
	Coin Rotation Task z-score		
DHA	-0.33 (1.17)	-0.24 (1.45)	-0.003 (1.44)
Placebo	-0.25 (1.18)	-0.45 (1.47)	-0.12 (1.46)
	COAST incongruent percentile score		
DHA	57.80 (22.99)	61.52 (22.65)	65.24 (21.54)
Placebo	53.68 (22.80)	62.59 (24.76)	64.75 (22.44)
	Digit Span Forward percentile score		
DHA	75.40 (24.31)	74.96 (27.67)	84.51 (19.14)
Placebo	63.25 (26.02)	73.26 (25.78)	77.12 (22.11)
	Digit Span Backward Test percentile score		
DHA	56.08 (32.45)	55.99 (34.53)	63.63 (31.08)
Placebo	49.53 (30.53)	47.51 (30.27)	51.39 (30.15)
	Digit Span Total scaled score		
DHA	12.36 (3.37)	12.37 (3.52)	13.6 (3.42)
Placebo	11.20 (3.17)	12.10 (3.53)	12.13 (3.00)
	Trail Making Test B percentile score		
DHA	41.95 (30.44)	41.47 (32.16)	48.01 (31.13)
Placebo	49.88 (31.57)	48.40 (31.62)	56.79 (32.30)

Note. [1] Values are means; SDs in parentheses

Table N2. Data for well-being/anthropometric measures at baseline, 6- and 12-months

Treatment group	Baseline[1]	6 months	12 months
	Geriatric Depression Scale (GDS) score[2]		
DHA	1.97 (2.01)	2.13 (2.38)	1.97 (2.74)
Placebo	2.17 (2.35)	2.43 (2.35)	2.13 (2.24)
	Geriatric Anxiety Inventory (GAI) score[3]		
DHA	2.23 (3.02)	2.53 (3.67)	1.83 (3.13)
Placebo	2.60 (3.64)	3.37 (4.19)	1.90 (2.36)
	SF-36 Physical functioning[4]		
DHA	50.04 (6.98)	49.31 (6.63)	50.07 (6.56)
Placebo	51.27 (5.90)	49.57 (6.34)	49.75 (6.42)
	SF-36 Role physical		
DHA	50.44 (6.85)	49.89 (6.89)	49.97 (7.33)
Placebo	50.04 (6.41)	50.49 (6.50)	48.99 (6.84)
	SF-36 Bodily pain		
DHA	50.48 (7.67)	49.90 (7.73)	52.12 (7.35)
Placebo	52.58 (8.87)	53.85 (6.24)	49.97 (8.10)
	SF-36 General health		
DHA	54.62 (6.82)	55.50 (5.47)	54.77 (6.73)
Placebo	54.31 (8.05)	54.42 (5.94)	54.91 (7.74)
	SF-36 Vitality		
DHA	56.06 (6.04)	54.98 (5.69)	56.46 (7.15)
Placebo	53.19 (5.59)	53.39 (8.47)	53.29 (9.01)
	SF-36 Social functioning		
DHA	52.32 (6.71)	53.33 (5.49)	53.49 (5.69)
Placebo	51.49 (6.04)	54.66 (4.31)	49.99 (9.29)
	SF-36 Role emotional		
DHA	51.06 (7.91)	50.83 (7.13)	51.99 (7.04)
Placebo	50.07 (7.74)	49.32 (7.84)	50.36 (7.83)
	SF-36 Mental health		
DHA	55.09 (7.59)	55.23 (6.35)	55.23 (7.72)
Placebo	53.31 (6.76)	54.36 (7.22)	54.09 (5.98)
	Systolic blood pressure (mmHg)		
DHA	145.90 (15.89)	144.47 (16.77)	147.73 (16.69)
Placebo	140.23 (21.91)	139.33 (31.75)	150.93 (25.06)
	Diastolic blood pressure (mmHg)		
DHA	83.67 (11.88)	82.00 (13.89)	84.00 (12.92)
Placebo	80.07 (13.77)	79.03 (12.98)	81.90 (12.13)
	Body Mass Index		
DHA	27.55 (4.16)	27.77 (4.45)	27.57 (4.42)
Placebo	26.01 (3.94)	26.31 (3.98)	26.05 (3.99)

Note. [1] Values are means; SDs in parentheses

[2,3] Lower GDS and GAI scores indicate an improvement

[4] Higher SF-36 scores indicate an improvement

Appendix O. Change in measures from baseline to 6 months

Table O. Change in cognitive, well-being and anthropometric measures by group from baseline to 6 months

	DHA group (*n* = 30)			Placebo group (*n* = 30)			DHA compared to Placebo		
	Baseline	6 months	Change	Baseline	6 months	Change	Difference	Treatment effect	
								p	η^2
RTot[1]	101.9 (11.9)	104.0 (11.9)	2.1 (7.2)	100.3 (11.9)	104.73 (11.5)	4.4 (7.3)	-2.3 (-6.1, 1.4)[2]	.26[3]	.02
RIM	104.8 (16.2)	108.6 (16.8)	3.7 (13.3)	105.3 (13.9)	109.4 (10.7)	4.1 (10.5)	-0.4 (-6.5, 5.8)	.96	.00
RVC	96.6 (9.6)	95.7 (8.9)	-0.9 (9.1)	92.9 (12.6)	94.6 (9.8)	1.7 (10.3)	-2.6 (-7.6, 2.4)	.21	.03
RL	103.9 (13.8)	100.4 (10.3)	-3.5 (13.3)	103.2 (12.8)	104.2 (8.2)	1.0 (12.5)	-4.5 (-11.2, 2.0)	.22	.03
RA	103.7 (13.8)	105.2 (13.8)	1.5 (10.2)	102.9 (13.3)	106.6 (15.6)	3.7 (11.0)	-2.2 (-7.6, 3.3)	.46	.01
RDM	98.2 (11.3)	105.5 (10.3)	7.3 (8.9)	98.3 (12.7)	103.1 (13.7)	4.8 (9.9)	2.5 (-2.4, 7.3)	.29	.02
CRT	-0.33 (1.2)	-0.24 (1.5)	0.09 (0.8)	-0.25 (1.2)	-0.45 (1.5)	-0.2 (0.9)	0.3 (-0.1, 0.8)	.14	.04
COA	57.8 (22.9)	61.5 (22.6)	3.7 (15.2)	53.7 (22.8)	62.6 (24.7)	8.9 (12.7)	-5.1 (-12.4, 2.0)	.25	.02
DSF	75.4 (24.3)	74.9 (27.7)	-0.4 (25.0)	63.2 (26)	73.3 (25.8)	10.0 (26.8)	-4.7 (-16.4, 6.8)	.13	.04
DSBT	56.1 (32.5)	56.0 (34.5)	-0.08 (20.9)	49.5 (30.5)	47.5 (30.3)	-2.0 (19.6)	1.9 (-8.5, 12.4)	.92	.00
DSTot	12.4 (3.4)	12.4 (3.5)	0.0 (2.6)	11.2 (3.2)	12.1 (3.5)	0.9 (2.5)	-0.9 (-2.2, 0.4)	.15	.04
TMTB	41.9 (30.4)	41.5 (32.2)	-0.4 (22.5)	49.8 (31.6)	48.4 (31.6)	-1.5 (38.1)	1.0 (-15.2, 17.2)	.86	.00
GDS	1.97 (2)	2.1 (2.4)	0.2 (1.7)	2.2 (2.4)	2.4 (2.4)	0.2 (1.8)	0.1 (-1.0, 0.82)	.87	.00
GAI	2.2 (3.02)	2.5 (3.7)	0.3 (2.2)	2.6 (3.6)	3.4 (4.2)	0.8 (4.3)	-0.5 (-2.3, 1.3)	.66	.00
SF36PF	50.0 (6.9)	49.3 (6.6)	-0.7 (5.6)	51.3 (5.9)	49.6 (6.3)	-1.7 (4.5)	0.9 (-1.6, 3.5)	.51	.00
SF36BP	50.5 (7.7)	49.9 (7.7)	-0.6 (8.7)	52.6 (8.8)	53.8 (6.2)	1.2 (7.9)	-1.8 (-6.2, 2.4)	.43	.01
SF36GH	54.6 (6.8)	55.5 (5.5)	0.9 (5.1)	54.3 (8.1)	54.4 (5.9)	0.1 (8.1)	0.8 (-2.7, 4.2)	.90	.00
SBP	145.9 (15.9)	144.4 (16.7)	-1.4 (15.6)	140.2 (21.9)	139.3 (31.8)	-0.9 (22.0)	0.5 (-9.3, 10.4)	.93	.00
DBP	83.6 (11.9)	82.0 (13.9)	-1.6 (11.7)	80.1 (13.7)	79.0 (12.9)	-1.0 (10.6)	0.6 (-5.1, 6.4)	.96	.00

Note. RTot = RBANS Total score, RIM = RBANS Immediate Memory, RVC = RBANS Visuospatial/Constructional, RL = RBANS Language, RA = RBANS Attention, RDM = RBANS Delayed Memory, CRT = Coin Rotation Task, COA = COAST incongruent score, DSF = Digit Span Forward, DSBT = Digit Span Backward Test, DSTot = Digit Span Total, TMTB = Trail Making Test B, GDS = Geriatric Depression Scale, GAI = Geriatric Anxiety Inventory, SF36PF = SF-36 Physical functioning, SF36BP = SF-36 Bodily pain, SF36GH = SF-36 General health, SBP = Systolic blood pressure, DBP = Diastolic blood pressure

[1] Values are means; SDs in parentheses [2] Values are means; 95% CIs in parentheses [3] *P* values were derived by using ANCOVA (adjusted for age, gender and years of education). None of the analyses violated Levene's test of equality of error variances.