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Samantha Gardener
Edith Cowan University

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Samantha Gardener
Edith Cowan University, s.gardener@ecu.edu.au

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Impact of Nutrition on Cognition and its Association with Blood and Brain Alzheimer Disease Related Biomarkers

Samantha Louise Gardener

BSc (Hons)

Supervisors: Professor Ralph Martins
Dr Stephanie Rainey-Smith
A/Prof Jennifer Keogh
Mr Kevin Taddei

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School of Medical Sciences

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Abstract

Alzheimer's disease (AD), the most common form of senile dementia, currently affects over 35 million people worldwide. While there is no cure or effective treatment, early intervention programs hold considerable promise. Following particular dietary patterns represents one potential intervention strategy accessible to all.

Results from previous studies investigating the association of diet, cognition and biomarkers of AD are inconsistent: Positive results have been reported (1-7), whilst others have shown no associations. Prior to this thesis, no study has assessed the relationship of four dietary patterns to cognition, blood-based and neuroimaging biomarkers of AD in a large highly-characterised ageing cohort. Participants drawn from the Australian Imaging, Biomarkers, and Lifestyle study of ageing, provided a fasting blood sample, underwent comprehensive neuropsychological assessment and neuroimaging at baseline, 18 and 36 month follow-up assessments, and completed a Cancer Council of Victoria food frequency questionnaire (used to construct dietary patterns) at baseline.

Chapter 3 explored the relationship between dietary pattern adherence and cognition. AD participants demonstrated reduced adherence to the 'healthy' Mediterranean (MeDi) and prudent diets, and higher adherence to the 'unhealthy' western diet and the inflammatory dietary index compared to cognitively healthy controls (HC). Longitudinal analysis conducted on individuals classified as HC at baseline proposes the importance of adhering to a 'healthy' dietary pattern such as the MeDi, with respect to reducing risk for cognitive decline: Executive function and visuospatial functioning appeared most susceptible to the influence of diet.

Chapter 4 investigated the potential mechanisms underlying the observed effects of dietary patterns on cognition. A lack of significant associations between the MeDi and western diet patterns and biomarker indexes of metabolic syndrome and cardiovascular disease risk, suggests that modulation of these factors may not underlie the effects of diet on cognition reported in Chapter 3.

Consistent with published literature, we found our western dietary pattern to be positively associated with levels of blood-based biomarkers of inflammation and the

reverse to be true of our MeDi and prudent diet patterns. Our inflammatory dietary index was also strongly positively correlated with levels of numerous inflammatory biomarkers. The strong associations observed suggest that interplay between diet and elevated chronic inflammation may contribute to the effects of diet on cognition described in this thesis.

Chapter 5 assessed the 'reliability' (similarity of 12 month dietary intake recalled on different occasions) and 'validity' (intake agreement between FFQ and a four-day weighed food record) of the online CSIROFFQ following addition of questions regarding foods of interest in AD research. Our results suggest that the modified CSIROFFQ is 'reliable' and a 'relatively valid' tool which provides acceptable assessment of long-term dietary intake in Australian older adults, particularly in the context of AD research.

To our knowledge, this is the first study extensively comparing MeDi, inflammatory dietary index, western and prudent diet patterns to cognition and biomarkers of AD in an elderly, well-characterised cohort. Our results combined with published data, suggest diet has a role to play in AD prevention; however, it is clear that the complex link requires further characterisation.

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Statement of candidature

I certify that this thesis does not, to the best of my knowledge and belief:

i. incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;

ii. contain any material previously published or written by another person except where due reference is made in the text of this thesis; or

iii. contain any defamatory material;

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My thesis comprises data from the AIBL study, which includes data collated from many researchers around Australia. A lot of hard work has gone into establishing the AIBL study as one of the leading cohorts in Alzheimer's disease, and it is with such a fantastic team that this is possible. I wish to express my gratitude to all members of the AIBL team, too numerous to mention here.

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Abbreviations

ABCA1 ATP-binding cassette transporter A1
AD Alzheimer's disease
ALA Alpha-linolenic acid
aMCI-s Amnestic single domain MCI
aMCI-m Amnestic multiple domain MCI
AMPK AMP-dependent kinase
APOE Apolipoprotein E (genotype)
ApoE Apolipoprotein E (protein)
APP Amyloid precursor protein
AIBL Australian Imaging, Biomarkers and Lifestyle study
Aβ Amyloid beta
B2M Beta 2 microglobulin
BACE1 Beta-site APP-cleaving enzyme 1
BBB Blood brain barrier
BMI Body mass index
CAA Cerebral amyloid angiopathy
CCVFFQ Cancer Council of Victoria food frequency questionnaire
CD40 Cluster of differentiation 40
CHD Coronary heart disease
CHIP Carboxyl terminus of the Hsc70-interacting protein
CI Confidence interval
COWAT Controlled Oral Word Association Task
CNS Central nervous system
CRP C-reactive protein
CSIRO Commonwealth Scientific Industrial and Research Organisation
CSIROFFQ CSIRO food frequency questionnaire
CSF Cerebrospinal fluid
CVD Cardiovascular disease
CVLT California Verbal Learning Test
C3 Complement component 3
DHA Docosahexaenoic acid
D-KEFS Delis-Kaplan Executive Function System
DLB Dementia with Lewy bodies
DNA Deoxyribonucleic acid
DSM-IV Diagnostic and statistical manual of mental disorders, 4th edition
EDTA Ethylenediaminetetraacetic acid
EGCG Epigallocatechin gallate
EGFR Epidermal growth factor receptor
ELISA Enzyme-linked immunosorbent assay
EOAD Early-onset Alzheimer's disease
EPA Eicosapentaenoic acid
FSANZ Food standards Australia New Zealand
FFQ Food frequency questionnaire
FSH Follicle-stimulating hormone
HC Healthy control
HDL High density lipoprotein
HDL-C High density lipoprotein-cholesterol
HIV Tat Human immunodeficiency virus transactivator of transcription
HMG 3-hydroxy-3-methylglutaryl
HR Hazard ratio
ICV Intracranial volume
ICD-10 International classification of disease, 10th revision
IDE Insulin degrading enzyme
IGF BP2 Insulin-like growth factor binding protein 2
IgM Immunoglobulin M
IL Interleukin
IL1ra Interleukin 1 receptor antagonist
IL-1β Interleukin-1β
IL Interleukin
LDL Low density lipoprotein

LH Luteinising hormone
LOAD Late-onset Alzheimer's disease
LM I Logical memory I
LM II Logical memory II
LMM Linear mixed model
MIF Migration inhibitory factor
MIP1α Macrophage inflammatory protein 1 alpha
MIP1β Macrophage inflammatory protein 1 beta
MPRAGE Magnetization Prepared Rapid Gradient Echo
MCI Mild cognitive impairment
MeDi Mediterranean diet
MMSE Mini-Mental State Examination
MRI Magnetic resonance imaging
MUFA Monounsaturated fatty acids
naMCI-s Non amnestic single domain MCI
naMCI-m Non amnestic multiple domain MCI
NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells
NFT Neurofibrillary tangles
NINCDS-ADRDA The National Institute of Neurological & Communicative Disorders & Stroke-Alzheimer's disease and related Disorders Association
NUTTAB95 Nutrient Tables for use in Australia 1995
NUTTAB 2007 Nutrient Tables for use in Australia 2007
OR Odds ratio
PA Physical activity
PATH Personality and Total Health
PET Positron emission tomography
PiB Pittsburgh compound-B
PGE1 Prostaglandin E1
PUFA Polyunsaturated fatty acid
PSEN1 Presinilin 1
PSEN2 Presinilin 2
RCFT Rey Complex Figure Test
ROI Region of interest
ROS Reactive-oxygen species
SD Standard deviation
SHBG Sex hormone-binding globulin
siRNA Silencing ribonucleic acid
SIRT1-7 Sirtuin (silent mating type information regulation 2 homolog) 1-7
SPSS Statistics Package for Social Sciences
SUV Standardised uptake value
SUVR Standardised uptake value ratio
TNFR1 Tumour necrosis factor receptor I
TNFR11 Tumour necrosis factor receptor II
TNFα Tumour necrosis factor alpha
TREM2 Triggering receptor expressed on myeloid cells 2
USA United States of America
VCAM1 Vascular cell adhesion molecule 1
VaD Vascular dementia
WAIS III Wechsler Adult Intelligence Scale (Ver III)
WMS Wechsler Memory Scale
24S-OHC 24S-hydroxycholesterol
cm Centimetre
GBq/µmol Gigabecquerel per micromole
g/d Grams per day
g/L Grams per litre
kCal Kilocalorie
kg Kilogram
kg/m^2 Kilograms per meters square
MBq Megabecquerel
mg Milligram
mm Hg Millimetres of mercury
mm/hr Millimetres per hour

mmol/L Millimoles per litre
mU/L Milliunits per litre
nM Nanometres
nmol/L Nanomoles per litre
pmol/L Picomoles per litre
U/L Units per litre
µg Microgram
µmol/L Micromoles per litre
y Years

Publications

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These manuscripts form the basis of Chapter 3.

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International conference proceedings

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Rainey-Smith S.R., Brown B.M., **Gardener** S., Peiffer J., Bourgeat P., Barnes M.B., Sohrabi H.R., Weinborn M., Taddei K., Rembach A., Laws S., Burnham S., Ellis K.A., Masters C.L., Macaulay S.L., Rowe C.C, Villemagne V., Ames D., Martins R.N/, For the AIBL Research Group. *APOE* genotype-dependent effects of diet and physical activity on cognition and Alzheimer's-related pathology: Data from the AIBL Study of Ageing. Oral presentation to the Alzheimer's Association International Conference for Alzheimer's disease, July 2014, Copenhagen, Denmark.

Chapter One

Introduction

Literature review

1.1. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of senile dementia, and it currently affects over 35 million people worldwide. Due to an increase in life expectancy and therefore an increase in ageing populations, this figure is predicted to increase to more than 115 million by 2050 (8).

In 1906, Alois Alzheimer was the first to report details of the symptoms together with the neuropathology of the dementing illness, which now bears his name. One of his patients, Auguste Deter, became ill in her 50's, her mental status deteriorated through a complex series of behavioural changes and cognitive decline, leaving her aggressive, delusional and unable to remember recent events. After her death, Alois Alzheimer performed an autopsy on her brain using recently developed staining methods, and discovered neuritic plaques as well as peculiar changes in the neurofibrils, now recognised as neurofibrillary tangles (9).

Neuritic plaques are extracellular deposits consisting mostly of aggregates of a 4 kDa peptide known as amyloid-beta (Aβ), whereas neurofibrillary tangles (NFT) are found intracellularly in neurons, and are composed of fibrils of abnormally phosphorylated tau protein. These two pathological findings, together with cerebral atrophy and widespread synaptic and neuronal loss are the pathological hallmarks of AD. Plaques and tangles are found in high frequency in the cortex and hippocampus, which is consistent with the behavioural changes seen in AD (10, 11). AD is a progressive neurodegenerative disease which develops over several decades, and initial symptoms include decline in cognitive function in domains including memory, executive function, language and visuospatial functioning, which is sufficient to interfere with everyday performance. Frequently there are also behavioural disturbances including depression, psychosis, anxiety, agitation, sleep disturbance, disinhibition and apathy (12). Recent post-diagnosis estimates of survival in AD range from three to nine years. The estimation of survival times using cohorts is problematic however, because of diversity in baseline disease severity: the inclusion of cases with advanced disease can lead to an underestimation of survival, while not including patients who die rapidly after diagnosis overestimates survival (13). There is no cure for the disease, and currently available treatments only reduce symptoms temporarily. It should be emphasised however, that the disease is believed to develop in the brain for two to three decades before symptoms

appear, providing a long time-frame for implementing any potentially preventative therapy or measures.

1.2. Forms of Alzheimer's disease

AD is broadly divided into early-onset AD (EOAD) and late-onset AD (LOAD). EOAD accounts for about 3% of all AD sufferers, it is often identified due to a family history of EOAD, and starts to affect people when they are less than 60 years of age. It is a fast-progressing, aggressive form of AD. Over 80% of those with EOAD have an autosomal dominant mutation in one of three genes; the amyloid precursor protein gene (APP; located on chromosome 21), the Presenilin-1 gene (PSEN1; located on chromosome 14) or the Presenilin-2 gene (PSEN2; located on chromosome 1); such cases are referred to as early-onset familial AD (14). The exact causes of non-familial cases of EOAD are not completely understood. LOAD accounts for the majority of AD cases, it is generally considered sporadic, and seen in patients above the age of 60 years; the causes are not known, but risk factors have been identified, the main ones being age and carriage of an Apolipoprotein E (*APOE*) ε4 allele (15).

1.3. Pathological hallmarks of Alzheimer's disease

1.3.1. Neurofibrillary tangles

Neurofibrillary tangles (NFT) are intraneuronal bundles of paired helical filaments (Figure 1.1; 16), composed of a microtubule-associated phosphoprotein known as tau. Tau normally enhances the polymerization of tubulin into microtubules and stabilizes these organelles in neurons. Tau becomes excessively phosphorylated, due to a combination of enhanced activity of certain kinases and decreased activity of certain phosphatases. Modification of this normally soluble neuronal protein into an insoluble filamentous polymer appears to involve a dysregulation of cytoplasmic phosphorylation/dephosphorylation cascades (17).

Defective proteosomal degradation of hyper-phosphorylated tau may contribute to the build-up of NFT. Tau interacts with the carboxyl terminus of the Hsc70-interacting protein (CHIP), an E3 ubiquitin ligase required for degradation of soluble phosphorylated tau. In AD, the mechanism of stabilisation and accumulation of hyper-phosphorylated tau may involve inhibition of tau interaction with CHIP. In addition to phosphorylation, tau is also acetylated; acetylation has been shown to impair the

proteosomal degradation and enhance the accumulation of tau (12). Recent advances in *in vivo* imaging of cerebral tau deposits utilising positron emission tomography (PET) will likely provide insight into the progression of tau pathology in AD (18, 19).

1.3.2. Neuritic plaques

Neuritic plaques are comprised of extracellular deposits of Aβ peptides, principally in a filamentous form (Figure 1.1). The Aβ peptides are cleavage products of the much larger membrane-spanning precursor protein already mentioned above – the amyloid precursor protein (APP). Aβ peptides vary in length, with the $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides of greatest interest in AD research. In the cerebrospinal fluid (CSF) of healthy controls, half of the Aβ ends at amino acid 40, 16% ends at amino acid 38, and 10% ends at amino acid 42 (20). APP is found ubiquitously in the body, yet the disease pathology is confined to the brain. The $A\beta_{1\text{-}40}$ species is normally more abundantly produced by cells than its aggregation-prone counterpart, $A\beta_{1\text{-}42}$ ($A\beta_{1\text{-}40}$ accounts for approximately 90% of all Aβ normally released from cells, and $A\beta_{1\text{-}42}$ approximately 10%), however much of the fibrillar Aβ found in the amyloid plaques is the 42 amino acid species ($A\beta_{1\text{-}42}$); this slightly longer, more hydrophobic form of Aβ is particularly prone to aggregation (21). $A\beta_{1\text{-}42}$ oligomers produced in the early steps of aggregation are believed to be the toxic principle behind the synaptic damage that occurs in AD, plaques and much larger aggregates are believed to be relatively inert (22).

Diffuse plaques are also present in the brain, which are comprised of $A\beta_{1\text{-}42}$. One hypothesis suggests that these diffuse plaques represent precursor lesions of neuritic plaques (21). Healthy aged individuals free of dementia often demonstrate solely diffuse plaques, in the same brain regions that AD patients show mixtures of diffuse and neuritic plaques; thus it is thought that the ratio of diffuse to neuritic plaques depends on the severity of disease. This theory is supported by transgenic mouse models of AD which show that diffuse deposits develop before fibrillar, thioflavin T-positive and congo red-positive neuritic plaques (21).

In vivo imaging utilising PET and radiolabelled amyloid ligands has enabled the time course of amyloid deposition in the brain to be studied: results suggest that deposition of amyloid in the brain commences 15 – 20 years before clinical diagnosis of AD (23).

Cerebral amyloid angiopathy (CAA), another major hallmark of AD, is characterised by Aβ accumulation in the cortical and leptomeningeal vessel walls (24). CAA is also known as congophilic amyloid angiopathy, as the amyloid aggregations can be stained by the Congo red dye. CAA is commonly associated with mutations in the APP gene within the Aβ sequence region of APP, at amino acid residue 693 (25).

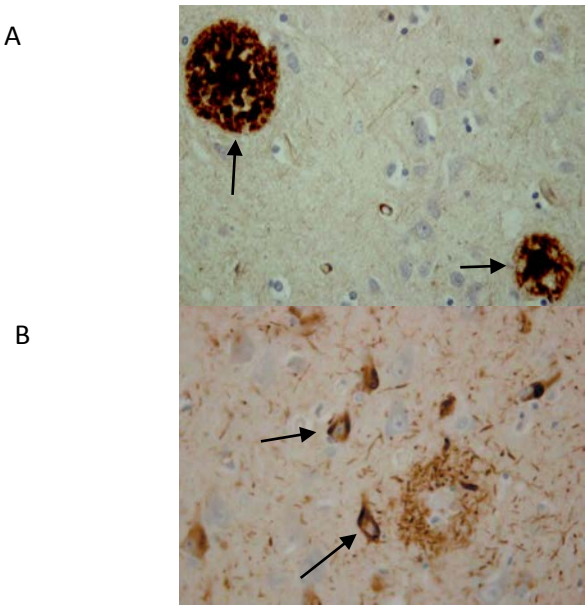

Figure 1.1. *Classic pathological hallmarks of Alzheimer's disease. (A) Neuritic plaques, shown by concentrated areas of brain staining and indicated by arrows; (B) neuritic plaque and neurofibrillary tangles, indicated with arrows. Adapted from Vandenberghe and Tournov (26).*

1.4. Amyloid beta and amyloid precursor protein

The Aβ protein is derived from the type I transmembrane protein APP by the amyloidogenic processing pathway involving two proteases. The APP gene (located on chromosome 21q21.2) is mutated in a significant fraction of familial AD cases and such mutations are associated with increased production of APP and alterations in the type of Aβ produced. Normal physiological function of APP is poorly understood, however punitive functions are proposed to include regulation of neuritic outgrowth, cell adhesion, synaptogenesis and cell survival (27-29).

APP can undergo amyloidogenic (produces Aβ) or non-amyloidogenic processing, depending on the proteases involved (see Figure 1.2). Amyloidogenic processing to produce Aβ from its precursor APP involves a two-step cleavage process. Firstly, the β-

site APP cleaving enzyme 1 (BACE1) cleaves APP in the luminal domain proximal to the transmembrane segment and thereby releases the N-terminal soluble portion of APP. Then the intramembrane γ-secretase enzyme mediates the subsequent cleavage at the C-terminus of Aβ, producing $A\beta_{1-40}$ or $A\beta_{1-42}$. As discussed in the previous section, the $A\beta_{1-40}$ form accounts for approximately 90% of all Aβ normally released from cells, and the $A\beta_{1-42}$ form accounts for approximately 10% of secreted Aβ, but is the predominant form found in amyloid plaques (30). Non-amyloidogenic processing involves α-secretase in place of BACE1. The α-secretase cleaves APP within the Aβ domain, so no Aβ is produced (see Figure 1.2).

Many factors have been shown to influence whether APP is processed via the amyloidogenic or the non-amyloidogenic pathway. These factors include cell membrane lipid composition, as APP cleavage enzymes (particularly γ-secretase which is found in membrane lipid rafts) is influenced by membrane lipid composition. Furthermore, it has been suggested that increasing α-secretase cleavage will reduce the production of Aβ, and α-secretase-mediated APP cleavage can be activated via several G protein-coupled receptors and receptor tyrosine kinases. For example, protein kinase C, mitogen-activated protein kinases, phosphatidylinositol 3-kinase, Cyclic adenosine monophosphate and calcium are activators of receptor-induced α-secretase cleavage (31).

Amyloidogenic versus non-amyloidogenic processing of APP is also influenced by other factors such as levels of cleavage enzymes in different cells, and levels of sex hormones. For example, amyloidogenic processing is predominant in neurons due to the high abundance of BACE1 in these cells (32), whilst oestrogen and testosterone have been shown to promote APP processing through the non-amyloidogenic pathway at the expense of the amyloidogenic pathway, resulting in the preclusion of Aβ generation (33).

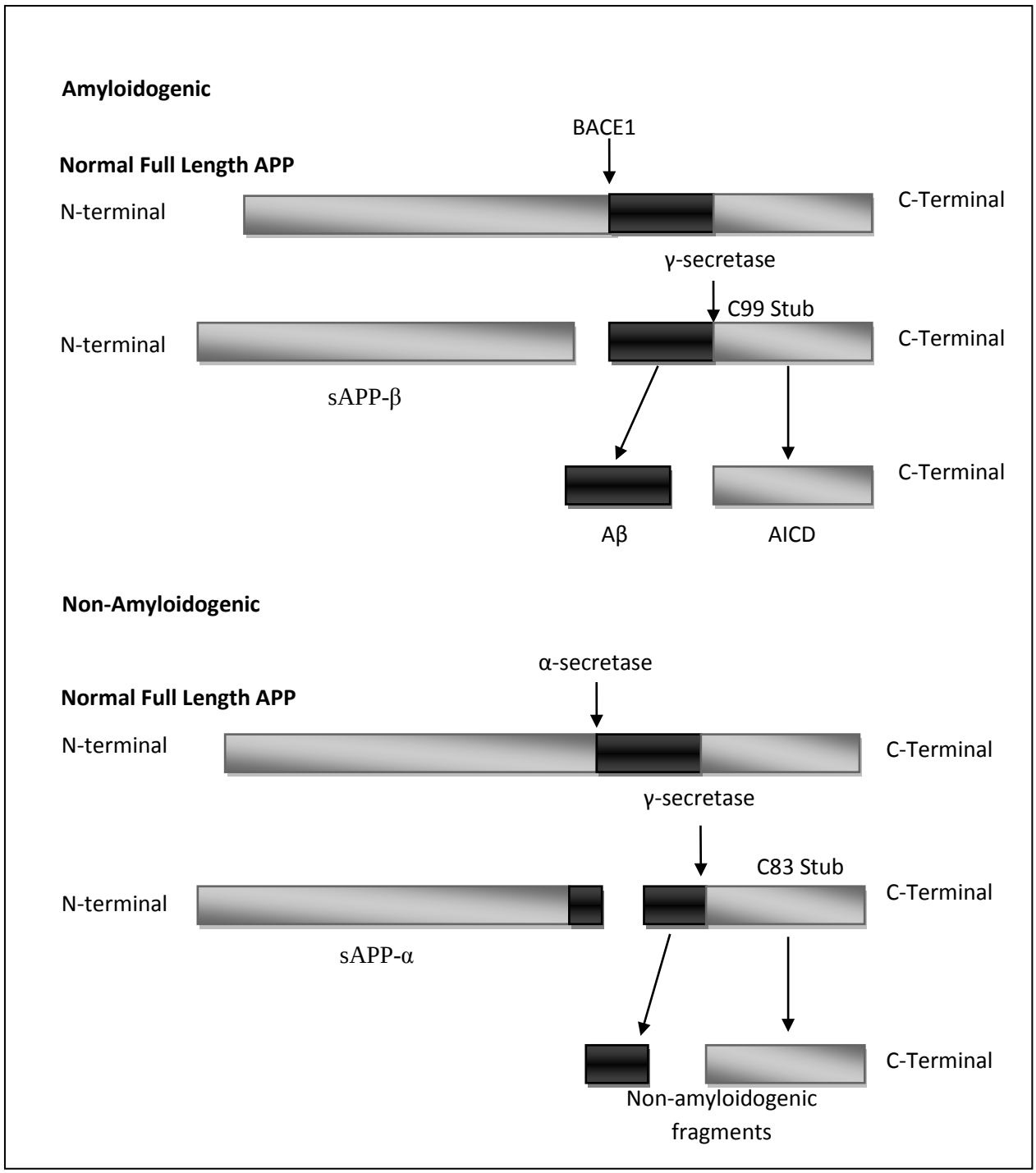

Figure 1.2: *Amyloidogenic and non-amyloidogenic APP processing pathways. Adapted from Verdile et al. (30).*

Abbreviations: AICD, APP intracellular domain; APP, amyloid precursor protein; Aβ, amyloid beta; BACE1, β-site APP cleaving enzyme 1; C83, non-amyloidogenic C-terminal fragment of 83 amino acid residues; C99, amyloidogenic C-terminal fragment of 99 amino acid residues; sAPP, soluble N-terminal fragment APP.

1.5. Amyloid cascade hypothesis

Amyloid plaques are large and highly insoluble, and as mentioned previously, they are believed to be relatively inert. There is mounting evidence that the much smaller oligomers of Aβ, particularly $A\beta_{1-42}$, are highly neurotoxic; these have been shown to induce free radical production, penetrate and damage cell membranes, and increase oxidative stress. In some other studies however, it has been found that the slightly larger aggregates - the Aβ-derived diffusible ligands (ADDLs - a neurotoxic subset of soluble $A\beta_{1-42}$ oligomers) are physiologically responsible for the memory malfunction and neurodegeneration seen in AD (22).

Nevertheless, there is abundant evidence that the overproduction, or the reduced clearance of $A\beta_{1-42}$ from the brain (normally by uptake and degradation in brain cells or by transfer to the periphery), together with oxidative stress, leads to inflammatory and further oxidative changes including mitochondrial dysfunction, eventually resulting in neuronal loss and associated cognitive decline. Some of the key evidence has arisen from studies on the effects of the EOAD-related mutations in cell culture models and *in vivo* in transgenic mice. These studies have indicated clearly that excessive levels of Aβ, or an increased proportion of Aβ being the longer $A\beta_{1-42}$ species, are implicated in causing EOAD.

The amyloid cascade hypothesis proposes a central role for Aβ overproduction or reduced clearance, followed by oligomerisation (and possibly fibrillogenesis) in triggering a cascade of cellular and molecular events, which in turn leads to the development of synaptic failure, synapse loss, neuronal death and eventually AD. This cascade is illustrated in Figure 1.3 (34, 35).

The hypothesis suggests that the dysregulation of APP processing occurs early in the disease process, resulting in the overproduction of $A\beta_{1-42}$. Small toxic oligomers of this Aβ are formed, causing membrane damage, oxidative stress and synaptic dysfunction. These small oligomers of Aβ can coalesce into fibrils and diffuse plaques, which can act as seeds for further deposition of Aβ. As plaques mature, the Aβ peptides reorganise into β-pleated sheets and form neuritic plaques. The formation of the neuritic plaques results in microglial and astrocyte activation, further oxidative injury, tau aggregation and phosphorylation, culminating in further synaptic dysfunction and neuronal loss, which eventually reaches a critical level and manifests as dementia (9).

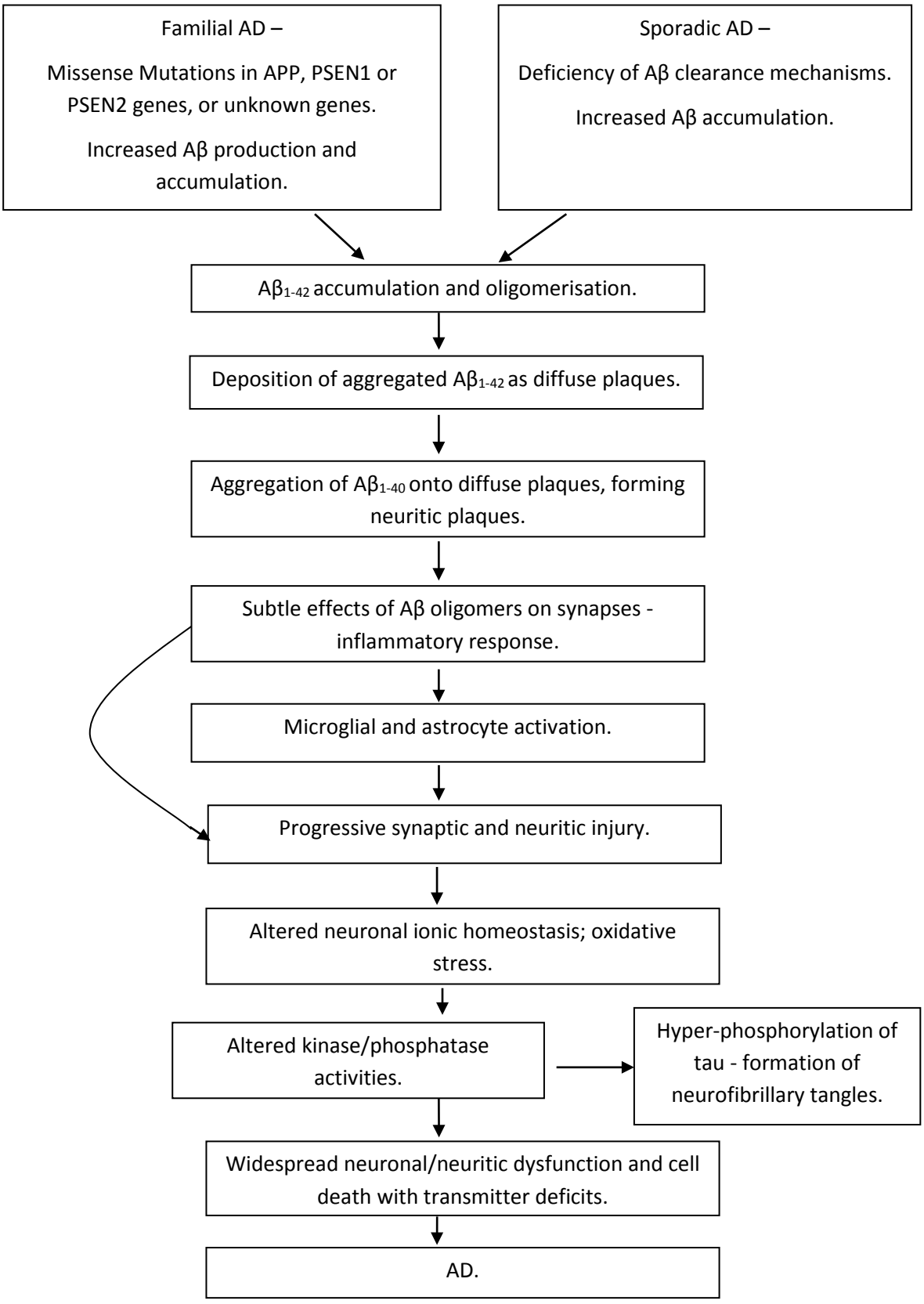

Figure 1.3: *The amyloid cascade hypothesis. Adapted from Herrup (9).*
Abbreviations: APP, amyloid precursor protein; Aβ, amyloid beta; $Aβ_{1-40}$, Aβ species ending in amino acid 40; $Aβ_{1-42}$, Aβ ending in amino acid 42; PSEN1, Presenilin-1; PSEN2, Presenilin-2.

1.6. Diagnostic strategies

1.6.1. Diagnostic criteria

Currently, a definitive diagnosis of AD requires an autopsy, to demonstrate the presence and characteristic pattern of distribution in the brain of the neuropathological lesions originally described by Alois Alzheimer: plaques and NFT.

Clinically, AD is most commonly diagnosed using one of the following three diagnostic criteria: International Classification of Disease, 10th Revision (ICD-10; 36); the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (37); and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA; 38). Clinical diagnosis requires the exclusion of other disorders that also contribute to deficits in memory and cognition, such as dementia with Lewy bodies (DLB) and fronto-temporal dementia (38). The criteria for the clinical diagnosis of AD have recently been updated to incorporate advances in our understanding of AD, advances in our ability to detect the pathophysiological process of AD, and the application of biomarkers for diagnostic purposes (see sections 1.6.4 and 1.6.5; 39).

1.6.2. Mild cognitive impairment

Mild cognitive impairment (MCI) is characterised by cognitive dysfunction at a level that is not of sufficient severity for a diagnosis of AD, and which does not significantly restrict activities of daily living (DSM-5; 40). MCI often, but not always, precedes AD. Studies have been contradictory concerning the percentage of MCIs that convert to AD, and the time frame in which this conversion occurs (40, 41). MCIs with higher cerebral brain amyloid load are significantly more likely to convert to AD than those with less amyloid load, with faster converters having higher amyloid levels at baseline than slower converters (42, 43).

There are several types of MCI: amnestic MCIs have an impaired memory, non-amnestic MCIs do not have impaired memory but have impairment in other domains, for example executive function, language or visuospatial functioning. These two groups are then divided into single or multiple domain; amnestic single domain (aMCI-s) refers to impairment in just memory, and multiple domain (aMCI-m) refers to impairment in memory and at least one other cognitive domain. Single domain non-amnestic (naMCI-s) refers to impairment in one non-memory domain, and multiple non-amnestic (naMCI-

m) refers to impairment in more than one non-memory domain. MCI can also indicate the development of other conditions such as vascular dementia (VaD), DLB and fronto-temporal dementia (44).

1.6.3. Neuropsychological assessment

Neuropsychological assessment aids in the diagnosis of AD by objectively establishing cognitive impairment from standardised tests. Neuropsychological testing may be conducted to determine more specifically the type and level of cognitive impairment being experienced, as well as determine which abilities are preserved. Therefore, neuropsychologists administer tests that have been developed through rigorous research in order to study people's short- and long-term memory, attention, concentration, reasoning, and ability to solve problems and learn new information. Test results are then compared to expected results of other individuals of the same age and education level, to determine whether a specific individual is impaired (45).

Cognitive impairment is diagnosed when two of the following are observed -

- Impaired ability to acquire and remember new information - symptoms include: repetitive questions or conversations, misplacing personal belongings (in unexpected places, such as a watch being placed in a fridge), forgetting events or appointments, getting lost on a familiar route.
- Impaired reasoning and handling of complex tasks, poor judgment - symptoms include: poor understanding of safety risks, inability to manage finances, poor decision-making ability, and inability to plan complex or sequential activities.
- Impaired visuospatial abilities - symptoms include: inability to recognize faces or common objects or to find objects in direct view despite good acuity, inability to operate simple implements, or orient clothing to the body.
- Impaired language functions (speaking, reading, and writing) - symptoms include: difficulty thinking of common words while speaking, hesitations, and speech, spelling, and writing errors.
- Changes in personality or behaviour - symptoms include: uncharacteristic mood fluctuations such as agitation, impaired motivation, initiative, apathy, loss of drive, social withdrawal, decreased interest in previously liked activities, loss of empathy, compulsive or obsessive behaviours, and socially unacceptable behaviours (46).

The clinical dementia rating (CDR) was developed for the evaluation of staging severity of AD. The CDR is a five-point scale in which CDR 0 implies no cognitive impairment, and then the remaining four points are various stages of AD (CDR 0.5 = very mild, CDR 1 = mild, CDR 2 = moderate and CDR 3 = severe). There are six domains used to construct the overall CDR – memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. In rating each of the domains, the assessment is on the patient's cognitive ability to function in these areas. The CDR is generated from a semi-structured interview with the patient and a knowledgeable collateral source (47).

1.6.4. Pittsburgh compound-B positron emission tomography

Extensive cognitive assessment of a person suspected of cognitive impairment clearly occurs only once symptoms are already present, and such assessment can only lead to a diagnosis of probable AD at best. The disease pathology, including Aβ aggregation, amyloid plaque deposition and NFT formation, is understood to develop for many years before a threshold level of damage is reached and symptoms appear. There is currently no cure or effective treatment for AD, despite at least three decades of extensive research into AD-specific potential treatments. As a result, current emphasis in treatment research is on preventative or disease-slowing therapy. For such treatments to be most effective, diagnosis at the earliest possible stage of the disease is necessary, thus the development of diagnostic methods that can detect the disease at early pre-clinical stages is of paramount importance. To this end, new Aβ-specific positron emission tomography (PET) radiotracers have been developed recently, which allow quantitative analysis of Aβ burden *in vivo*.

A PET scan is a sensitive molecular neuroimaging technique that uses radiolabelled tracers to evaluate biological processes *in vivo*, PET may permit detection of disease processes at asymptomatic stages when there is no evidence of anatomic changes using magnetic resonance imaging (MRI; 16). PET is capable of assessing brain function as well as structure and has proven even more powerful as a diagnostic tool compared with structural imaging techniques such as MRI.

For the purposes of detecting cerebral Aβ burden in AD, the radiolabelled tracer or ligand must be a small molecule capable of crossing the blood-brain barrier (BBB), and capable of binding to Aβ in a specific and selective fashion (16). Several radioligands have been trialled for this purpose, and to date, the best radioligand that has been developed is Pittsburgh compound-B (PiB; Figure 1.4). This ligand has high affinity and high specificity for amyloid fibrils and binds to amyloid plaques (48). PiB is a carbon-11-labelled derivative of the thioflavin-T amyloid dye (49). The molecular probes are firstly produced with positron emitting isotopes, and the imaging procedure begins with injection of the radioactive tracer into the patient. After a short time-span to allow for the probes to bind to amyloid, their distribution in the brain is determined using PET scanning.

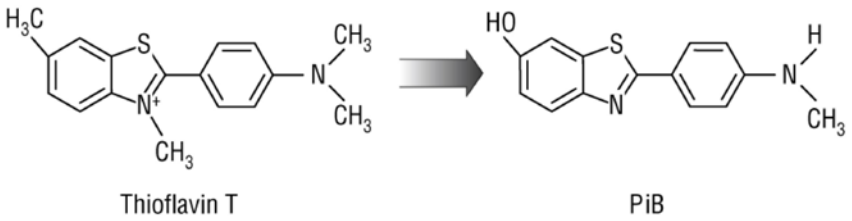

Figure: 1.4: *Derivation of Pittsburgh compound-B from the fluorescent histochemical dye thioflavin T (50).*

In a study of PiB PET by Klunk *et al.* (51) both imaging and time-activity curves showed that the retention of PiB provided a quantifiable discrimination between most mild AD patients and cognitively healthy controls (HC). In HC participants there was very little retention of PiB in cortical regions. On the other hand, frontal and temporoparietal cortical areas in AD patients, known to contain amyloid deposits, showed preferential retention of PiB. In contrast, the absolute level of PiB retention was approximately the same in the cerebellum and white matter of AD and HC subjects - brain areas known to lack substantial deposition of fibrillary amyloid. The absolute amount of PiB retained in the frontal cortex of AD patients was greater than 90% higher than that retained in the frontal cortex of HC subjects. This pattern of PiB retention is generally consistent with the pattern of amyloid plaque deposition described in *post mortem* studies of AD brain tissue (51).

One potential and highly desired application of PiB PET imaging is to identify the neuropathological manifestations associated with AD in clinically 'normal' individuals prior to the development of cognitive changes. These individuals would be considered

to have preclinical AD, and thus would be candidates for disease-delaying therapy. In one study, Rowe *et al.* (48) showed cortical PiB binding in 22% of the HC participants. This percentage is similar to the expected proportion of the general population that will develop AD by the age of 85 years. However, Rowe *et al*. (48) also showed that Aβ burden, as measured by PiB PET, does not correlate well with disease severity; yet there is increased Aβ in all AD subjects regardless of severity, supporting the concept that Aβ deposition occurs before the onset of cognitive symptoms**.** Therefore, PiB PET not only shows great promise for accurate early diagnosis of AD, but may also be able to detect preclinical AD. Other studies supporting this concept have shown extensive uptake of PiB in neocortical areas, in asymptomatic elderly subjects with documented recent decline in cognitive tests (16). Furthermore, studies of EOAD have found that autosomal dominant AD mutation carrier individuals have elevated PiB levels in nearly every cortical region 15 years before the estimated age of onset – supporting the potential of PiB PET in preclinical diagnosis (52). However, as in the study by Rowe *et al.* (48), other studies have also found that there is wide individual variation in the brain amyloid load in MCI, and in the course of amyloid accumulation in relation to the clinical diagnosis of AD (53).

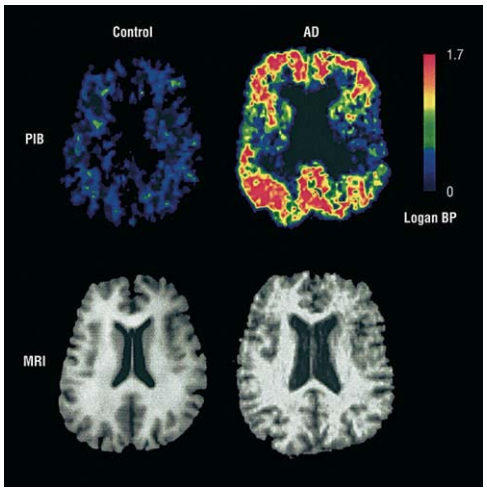

Figure 1.5: *Binding potential of Pittsburgh compound-B (PiB). From Mathis et al. (50). Sample brain images depicting the binding potential of PiB (top row) measured in a 70 year old healthy control and a 75 year old person with Alzheimer's disease (AD). Also shown are the corresponding anatomical magnetic resonance images (MRIs: bottom row). Greater retention of PiB is evident in frontal, temporoparietal, and posterior cingulate cortices of the AD individual, relative to the control. Logan BP indicates Logan binding potential (50).*

In a cohort of 206 individuals from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of ageing, 98% of participants with AD, 69% of MCIs and 31% of HCs showed high PiB retention. After a mean follow-up of 20 months, small but significant increases in PiB standard uptake value ratios (SUVRs) were observed in those with high PiB retention at baseline. Increases were associated with the number of *APOE* ε4 alleles (known AD risk factor – see section 1.7.1 below) carried by these individuals. Progression to AD occurred in 67% of MCIs with high PiB, versus 5% of those MCIs with low PiB, but 20% of the low PiB MCI subjects progressed to other dementias. Of the high PiB HCs, 16% developed MCI or AD by the 20 month follow-up and 25% by three years. Other studies have also found that Aβ deposition increases slowly from cognitively normal to moderate severity AD, that this deposition precedes cognitive impairment, and is associated with the *APOE* genotype (43).

Chételat *et al*. found a specific relationship between episodic memory deficits and neocortical temporal PiB, independent from hippocampal atrophy, in the predementia stage of the disease, suggesting a relationship between regional Aβ deposition and episodic memory deficits in the presymptomatic stage of AD (54). Interestingly, Pike *et al.* found increased Aβ was related to worse episodic memory and visuospatial performance in males but not females in a sample of 177 healthy older adults (55).

PiB has a short half-life of only 20 minutes; this reduces its practical application due to the need for onsite synthesis capabilities (cyclotron) which are only possible in specialised research centres. This makes PiB PET very difficult to adapt to general diagnostic usage. Other amyloid ligands utilising Fluorine-18 (^{18}F), including florbetaben, florbetapir and flutemetamol, are currently being tested. Although their pharmacodynamic properties are generally less favourable than those of PiB, they are nevertheless sensitive in the detection of brain amyloid *in vivo*, and their longer half-life (110 minutes) would increase imaging capabilities (56).

Despite the aforementioned limitations, the benefits of neuroimaging include improvements in early AD diagnosis with a presymptomatic biomarker, improved understanding of the natural history of amyloid deposition and insights into the pathophysiology of AD as well as the capability to measure effects of newly developed anti-amyloid therapies (50). The development of a more widely accessible neuroimaging technology will also be invaluable: when a treatment becomes available for AD, the ability to identify patients early in the disease process or pre-symptomatically using amyloid imaging will become essential for early and optimal initiation of therapy.

1.6.5. Magnetic resonance imaging

Data obtained from MRI scans can be merged with PET images, providing combined structural/functional information (57). Structural MRI provides visualisation of the macroscopic tissue atrophy that results from the cellular changes underlying AD. Recent advances in image analysis algorithms have led to the development of structural MRI-based software tools that can automatically divide the brain into anatomic regions and quantify the tissue atrophy in these regions for a single individual (58).

AD is significantly associated with cortical grey matter loss and ventricular enlargement. From MRIs, the severity of volume loss of the entorhinal, perirhinal and temporal cortices in patients with AD, has been shown to correlate with disease severity; these areas have also been shown to be reduced in both MCI and AD individuals compared to HCs (16). In a study of 112 cognitively HC, 226 MCI and 96

AD patients, each of whom had undergone at least three MRI scans, it was found that MCI and AD groups showed hippocampal volume loss over six months and accelerated loss over one year, when compared to HCs. Increased rates of hippocampal loss were also associated with the presence of *APOE* ε4 alleles (59).

Chételat *et al.* (60) published findings on the relationship between atrophy (measured by MRI) and Aβ deposition (measured by PiB PET). They found a strong relationship between Aβ deposition and atrophy very early in the disease process (in those with subjective cognitive impairment) but not in patients with MCI or AD, suggesting that anti-amyloid therapy should be administered early in the disease process to minimise neuronal loss.

1.7. Alzheimer's disease risk factors

1.7.1. Apolipoprotein E[1]

The human Apolipoprotein E (*APOE*) gene is located on the long arm of chromosome 19 (19q13.2). There are three *APOE* alleles, denoted as *APOE* ε2, *APOE* ε3 and *APOE* ε4. The distribution of the three alleles in the general Caucasian population is approximately 8%, 78% and 14% for *APOE* ε2, *APOE* ε3 and *APOE* ε4 respectively (61). The apoE protein exists as three major isoforms, denoted apoE2, apoE3 and apoE4, with single amino acid substitutions of cysteine/arginine at residues 112 and 158 accounting for their differences (62).

It has been shown that *APOE* ε2 alleles may be associated with a delayed age of onset of AD, even in carriers of APP mutations. These findings have led to the proposal that the *APOE* ε2 allele may offer a level of protection against the development of AD (63). Conversely, the *APOE* ε4 allele is over-represented in sporadic and familial AD cases; it is considered the most common genetic risk factor for AD, and is present in over 50% of LOAD cases (63). Several studies have shown that the risk of AD increases as a function of the *APOE* ε4 allele dosage. This implies that APOE alleles in the order of ε4/ε4 > ε4/ε3 > ε4/ε2 or ε3/ε3 > ε3/ε2 > ε2/ε2 positively influence an individual's risk of developing AD (64, 65). However, carrying an APOE ε4 allele does not imply one is guaranteed to develop AD, and similarly, nor does it imply that individuals without *APOE* ε4 alleles are not at risk, they may nonetheless develop AD, though their risk is lower than average (66).

[1]When referring to Apolipoprotein E as a gene '*APOE*' will be used, when referring to apolipoprotein E as a protein 'apoE' will be used.

ApoE is one of several different classes of apolipoproteins, including apoD and apoJ, which transport lipids in plasma and CSF. ApoE is one of the most important cholesterol transport proteins, with peripheral apoE mainly synthesised in the liver, whilst other major sources include tissue macrophages. It is the major lipoprotein within the CNS, where it is synthesised and secreted from astrocytes and microglia (67). Carriage of the *APOE* ε4 allele is linked to enhanced amounts of diffuse $A\beta_{1-42}$ plaques in the brain, and to an earlier age of onset of the disease. The mechanism by which the apoE4 protein leads to these differences has been researched extensively, yet is still not well understood (68). ApoE appears to be necessary for amyloid deposition, as apoE knockout mice crossed with AD-model mice deposit considerably less amyloid (69, 70) and when compared to apoE3, apoE4 promotes greater deposition of Aβ peptides. These studies show that apoE assists Aβ deposition, and that apoE4 may increase AD risk by increasing levels of amyloid deposition. However, apoE also appears to be involved in the binding and clearance of brain Aβ, with apoE3 and apoE2 being more effective than apoE4. ApoE3 interactions with Aβ oligomers and apoE receptors also reduce the neurotoxicity of Aβ oligomers, and to a greater extent than apoE4, again possibly via better clearance (71). In other studies, apoE4 has been found to be less protective against oxidative stress, to affect signalling pathways, and to result in greater cholinergic dysfunction in AD (72).

1.7.2. Age

The greatest known risk factor for AD is advancing age (Figure 1.6). Most individuals with the disease are aged 65 years or older and the likelihood of developing AD doubles approximately every five years after the age of 65. After the age of 85, the likelihood of developing AD reaches nearly 50 percent (73, 74). Due to increased longevity, as a result of improved healthcare, the prevalence of AD is increasing.

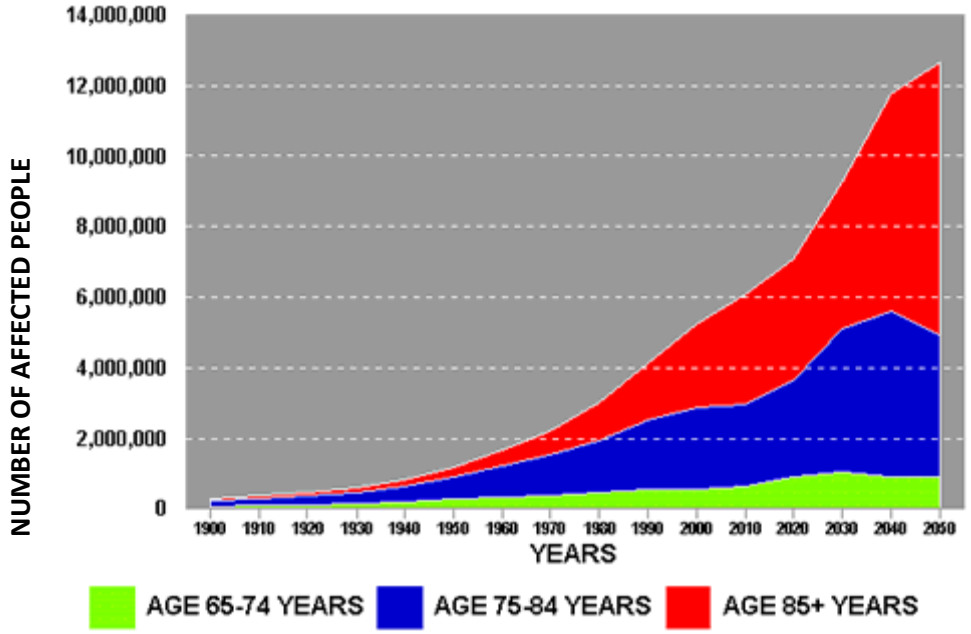

Figure 1.6: *Prevalence of Alzheimer's disease currently in America, and a projection of the number by 2050. Graph courtesy of University of Oklahoma, 2012.*

1.7.3. Cardiovascular disease

There is a large body of evidence which suggests that many cardiovascular disease (CVD) risk factors directly influence AD risk (75, 76).

1.7.3.1. Obesity

Mid-life obesity, which is associated with altered cholesterol and lipid metabolism, has been associated in a number of longitudinal studies with later life dementia risk (77-81). Some studies have concluded the opposite, for example Fitzpatrick *et al.* (82) reported an inverse association with mid-life (50 years) obesity and dementia risk; however, this association became non-significant in late-life (65 years and older) in their study. Other studies have indicated that this association is reversed in adults over 70 years of age, with lower body fat a predictor of cognitive decline (83).

Obesity and its association with AD have been linked to abnormal metabolism of the obesity-related protein leptin. For example, weight loss often precedes the onset of AD, and there is a correlation between reduced levels of circulating leptin and the risk of

AD; lower levels of circulating leptin have been observed in AD patients versus HCs (84). A large prospective study involving 2871 elderly people followed over a period of four years showed that lower leptin levels were associated with greater cognitive decline (85).

Leptin is a peptide hormone secreted by adipose tissue; it exhibits a wide range of central and peripheral actions, including providing signals to specific regions of the hypothalamus to control energy homeostasis, thereby leading to fat storage or mobilisation which also controls insulin sensitivity (86). Another activity of leptin is the control of AMP-dependent kinase (AMPK); AMPK maintains lipid levels and also regulates glycogen synthase kinase-3 which modulates tau phosphorylation (87). Furthermore, leptin has been shown to suppress Aβ generation *in vitro* by attenuating BACE1 processing of APP in neuronal cells, possibly through mechanisms involving altered lipid composition of membrane lipid rafts. The chronic administration of leptin to AD-model transgenic mice reduced the brain Aβ load and tau phosphorylation in neuronal cells that normally develops in these mice, suggesting leptin has potential as a therapeutic factor (88). Leptin appears to be regulated in humans and animals by short-term alterations in energy intake, as leptin declines with fasting and increases with acute overfeeding (89). It has been shown that leptin levels significantly correlate with levels of certain dietary variables, particularly dietary fat levels and inversely with dietary carbohydrate levels, but show no correlation with protein levels in the diet or total energy intake (90).

1.7.3.2. Cholesterol

AD patients have been shown to have a lipoprotein profile commonly found in atherosclerosis patients; as they frequently demonstrate increased levels of total plasma cholesterol and low density lipoprotein (LDL) as well as reduced levels of high density lipoproteins (HDL), when compared to age-matched controls (91). Disturbances in cholesterol homeostasis are a hallmark of neurodegenerative diseases. Statins have been used to lower cholesterol levels in coronary artery disease, and some studies suggest they may protect against AD (91-93), although not all studies agree, partly due to the different statins available, and due to patient age differences at the time of treatment. Statins work by inhibiting 3-hydroxy-3-methylglutaryl reductase (HMG reductase), which is the rate-limiting enzyme in cholesterol synthesis (67). Interestingly, levels of

total cholesterol and LDL in serum correlate with Aβ load in the brains of patients with AD, and epidemiological evidence suggests individuals with elevated cholesterol levels during mid-life have a higher risk of developing AD later in life (67).

The brain is the most cholesterol-rich organ in the body; it contains approximately 25% of total body cholesterol but is only 2% of total body weight. In the human brain, cholesterol is a major component of myelin and of neuronal and glial membranes. Nearly all brain cholesterol is synthesised *in situ*; virtually no cholesterol crosses the BBB. The brain can't degrade cholesterol, any excess is converted to 24S-hydroxycholesterol (24S-OHC), which can readily cross the BBB. This allows the exit of approximately 6 – 7 mg of cholesterol from the brain each day, with the liver then being responsible for the removal of the 24S-OHC (67). CSF 24S-OHC has been found to be higher in AD individuals, suggesting that cholesterol metabolism in CSF is disturbed in AD; cholesterol efflux also appears to be elevated and related to the increased risk for AD. It has been suggested that the increased levels of 24S-OHC possibly reflect neuronal cell death, with release of cell membrane cholesterol. Other factors that affect cholesterol equilibrium include disturbances and degeneration of the BBB which occur as part of the neuropathology occurring in AD brains (91). Furthermore, CVD-related reduced blood flow to the brain may also cause damage to the BBB, which adds to the already present changes in cholesterol and lipid metabolism.

There are three major categories of brain lipids: glycerophospholipids, sphingolipids and cholesterol. Sphingolipids and cholesterol self-aggregate into specific membrane domains known as lipid rafts, and these are involved in cellular trafficking and signalling functions. They are also preferential sites for host interactions with pathogens and toxins (94). Cholesterol can bind directly to APP, stimulating insertion into phospholipid monolayers, cholesterol also binds to Aβ protofibrils. However, whether cholesterol accelerates or decreases Aβ polymerisation is still uncertain. The generation of Aβ peptides through APP proteolysis occurs within lipid rafts and this proteolysis is sensitive to inhibitors of cholesterol biosynthesis (94). High cholesterol intake has been linked with AD-like pathology in animal models such as rabbits, in which diet-induced hypercholesterolemia is associated with increased Aβ levels and Aβ plaque load in the brain (75).

Increased LDL and decreased HDL (a form of dyslipidemia) are independent risk factors for coronary heart disease (CHD) and carotid artery atherosclerosis, which in turn leads to cognitive impairment through cerebral hypoperfusion or embolism (95). HDL-cholesterol (HDL-C) is believed to influence small-vessel disease by playing a protective role in the removal of excess cholesterol from the brain by interaction with apoE and heparin sulphate proteoglycans in the subendothelial space of cerebral microvessels (96).

A study by Ward *et al.* (97) found that grey matter volume within the right middle temporal gyrus was significantly associated with certain neuropsychological test results, suggesting that adults with decreased levels of HDL-C may be experiencing cognitive changes and grey matter reductions in regions associated with neurodegenerative disease, and therefore may be at greater risk of future cognitive decline. HDL-C has shown anti-inflammatory (98) and antioxidant effects (99), which may also help explain its protective effect against AD. Lifestyle interventions proven to increase HDL-C levels, for example healthy diet, exercise and weight control have also been shown to provide neuroprotective effects (97).

Another mechanism through which HDL may be able to confer a degree of protection against AD is through its ability to bind to Aβ, thereby maintaining its solubility in CSF (in HDL-like particles) and plasma, and preventing the deposition of Aβ in the brain. ApoE plays a major role in the regulation of lipid and lipoprotein (including HDL) homeostasis, and facilitates the transport of cholesterol to the liver where it is degraded. Interestingly, the apoE2 and apoE4 isoforms have been shown to influence HDL-C levels in opposite directions; apoE4 adversely affects the HDL-Aβ interaction (100).

1.7.3.3. Atherosclerosis

Atherosclerosis, a major degenerative disease of arteries, involves a series of inflammatory and oxidative modifications within the arterial wall. A study by Dolan *et al.* (101) examining data from 200 autopsies, found that atherosclerosis and dementia are related, however this only applied to intracranial atherosclerosis and not cardiac or aortic atherosclerosis. A study by Itoh *et al.* (102) found no association between AD pathology and aortic, cardiac or intracranial atherosclerosis, whilst Beeri *et al.* (103) found a relationship between neuritic plaques and coronary atherosclerosis, mostly in *APOE* ε4 allele carriers, using 99 brain autopsies of which 36 were *APOE* ε4 allele

carriers. The significance of these studies is limited because of relatively small sample sizes; there is a need for further larger studies to address inconsistent results. In a study of 4971 subjects, those with overt or clinically silent vascular disease performed worse on cognitive tests than those subjects without vascular pathology. Indicators of atherosclerosis of the carotid arteries and presence of atherosclerosis of the large vessels of the legs were associated with AD (104). The authors also reported that the prevalence of AD increased with the degree of atherosclerosis, and subjects with at least one *APOE* ε4 allele and severe atherosclerosis had nearly 20-fold increased risk for AD (104).

1.7.3.4. Hypertension

Hypertension affects 25% of the adult population and 50% of people over 70 years (105), it is diagnosed when systolic blood pressure exceeds 140 mm Hg, and/or diastolic pressure exceeds 90 mm Hg. Hypertension impairs the process by which brain activity and blood flow are coordinated (106). Hypertension is one of the most important risk factors for AD, stroke and CVD. It has been shown that hypertension precedes dementia onset by approximately 30 years, however this relationship is complex and does not follow a linear progression. Mid-life hypertension is particularly associated with an increased risk of developing AD, whereas elevated blood pressure late in life does not appear to have the same associated risk (107). MRI investigations have demonstrated that individuals with high blood pressure often have large areas of white matter hyperintensities, ventricular enlargement and silent infarcts, all of which can lead to cognitive dysfunction and dementia (108). In a longitudinal study, it was concluded that both high systolic and high diastolic blood pressure were associated with AD onset 10 to 15 years later. Individuals with hypertension also exhibit higher than average amounts of senile plaques and neurofibrillary tangles. However, the findings in the literature are contradictory, with both high and low blood pressure being associated with cognitive impairment, and some studies have reported no association. A possible explanation is that high blood pressure does indeed increase risk of AD over a long period, but in the years preceding onset, blood pressure starts to decline and declines further with progression of the disease (104). Clearly more prospective studies with a longer follow-up are required. Anti-hypertensive treatments have been associated with lower incidence of clinically diagnosed AD and better cognitive function, however there are several different antihypertensive medications, and not all of these have been found to have an effect on AD. Also, several investigators failed to find treatments effective in

reducing late-life AD. This could be due to differing methodology between studies, including differences in estimates of prevalence and incorrect diagnosis of dementia types, in particular vascular dementia (109). Larger studies utilising a greater age range of participants may circumvent these limitations.

1.7.4. Metabolic syndrome - Diabetes

High caloric intake and diets high in sugar and refined flour are major health concerns in the Western diet. Unhealthy lifestyle choices have led to a growing incidence of obesity and altered insulin receptor signalling due to hyperglycaemic conditions (88), this can often lead to type-2 diabetes. It has been reported in several studies that type-2 diabetes (characterised by resistance to the effects of insulin) is associated with a two- to three-fold increased relative risk for AD (110-114). Abnormalities in insulin metabolism in diabetic conditions are thought to impact the onset of AD partly via their influence on the synthesis and degradation of amyloidogenic Aβ peptides. For example, insulin itself may significantly promote Aβ accumulation by accelerating APP/Aβ trafficking from the trans-Golgi network, a major cellular site for Aβ generation, to the plasma membrane. Elevated levels of circulating insulin in diabetic conditions may also provoke amyloid accumulation by directly competing with Aβ for the insulin-degrading enzyme (IDE), thereby limiting Aβ degradation by IDE (115).

Recent evidence suggests a role for insulin in normal memory function, supporting the hypothesis that insulin by itself affects many mechanisms relating to neuronal activity and cognitive function (88). Type-2 diabetes and AD both have significant inflammatory components, and there is evidence that inflammation based mechanisms in type-2 diabetes might help induce the neuroinflammation and microglial activation that occurs in AD (116). However, not all studies support the hypothesis that diabetes increases risk of AD (117-119). Arvanitakis *et al.* (120) reported that diabetes is associated with a lower level of semantic memory but not with other cognitive domains. There are factors within such studies which could provide an explanation as to why some found a significant association and others did not. The reporting of diabetes almost certainly underestimates the true prevalence of the condition; true prevalence is believed to be twice that of self-reported disease. Another possible explanation is the potential mis-classification of AD which would cause an underestimation of the relationship between AD and diabetes: the studies use a variety of criteria to diagnose

AD which may lead to misclassification of vascular dementia or stroke-associated dementia as AD. This again highlights the need for better diagnostic methods.

1.8. Lifestyle risk factors

There is currently no cure in sight for AD, and for this reason much attention has shifted to identifying factors or treatments which may delay the onset or slow disease progression. Earlier in this chapter we reported neuropathological changes (specifically cerebral Aβ deposition) that precede onset of symptoms by 15 – 20 years, thereby presenting a 'window of opportunity' to modify disease course. There is significant benefit to ascertaining which lifestyle factors may protect against or contribute to the development of AD. The identification of such factors might permit early intervention to modify disease course, to delay or defer the onset of AD (11). Physical activity, smoking, and diet represent examples of lifestyle risk factors. Modification of these lifestyle factors is accessible to all, and economically advantageous over medical interventions with reduced incidence of adverse side effects.

1.8.1. Physical activity

1.8.1.1. Effects of physical activity

It is common knowledge that regular physical activity (PA) should be part of a healthy lifestyle in all age groups. Benefits include reduced mortality, prevention of CVD, positive effects on lipid profiles, successful weight management; the prevention and management of type 2 diabetes, hypertension, stroke and certain cancers; also a reduced risk of falls and bone fractures, and potentially an enhancement of cognitive function (121).

In general, exercise has been shown to have positive effects on many aspects of cognition such as reasoning, working memory, executive functioning, vigilance monitoring and fluid intelligence test performance (122). PA is a complex behaviour characterised by intensity, duration and frequency. Discrepancies between studies on PA could be caused by differences in assessment methods and measurement, for example, some studies use only self-reported PA questionnaires, whereas others use accelerometers to measure daily PA. Accelerometers provide an objective measure of habitual activity which is not dependent on self-reporting (123). Results from 217

participants of the AIBL study have shown that intensity, rather than volume, of PA is associated with cognitive functioning. Compared with individuals in the lowest tertile of PA intensity, those in the highest tertile scored 9%, 9%, 6% and 21% higher on the digit span, digit symbol coding, Rey Complex Figure Test copy and 30 minute delay recall, respectively (124). A study by Scarmeas *et al.* (125) suggested that increased PA is associated with a reduced risk for developing AD, and the higher the intensity, frequency and duration of the PA, the more the decrease in risk, with a dose-dependent response observed. In Scarmeas' study, high PA corresponded to approximately 1.3 hours of vigorous PA per week, or 2.4 hours of moderate PA per week, or 4 hours of light PA per week; even this relatively 'small' amount of PA was associated with a reduction in risk for developing AD.

1.8.1.2. Possible mechanisms of action of physical activity

There are many possible mechanisms that may explain the protective effect of PA in relation to AD risk, one of these mechanisms is angiogenesis; exercise promotes vascular antioxidant pathways, capillary growth and improves cerebral perfusion (126). Chronic exercise could maintain cerebrovascular integrity by enhancing oxygen transportation to the brain and this enhanced blood flow may positively influence cognitive performance (127). PA also enhances synaptic connections between brain cells and influences neuronal plasticity (128). Prolonged PA has also been shown to maintain neurogenesis and to slow age-associated cognitive decline (129). Exercise has been shown to induce hippocampus hypertrophy - exercise-induced production of growth factors such as the brain-derived neurotrophic factor (BDNF) has been shown to enhance neurogenesis and to play a key role in positive cognitive effects. There is evidence that insulin-like growth factor (IGF-1) may mediate the exercise-induced changes in BDNF, neurogenesis, and cognitive performance. IGF-1 is also postulated to regulate brain Aβ levels by increased clearance via the choroid plexus (130). Studies of transgenic mouse models of AD have also shown that exercise causes a host of beneficial changes, including repression of neuronal cell death, and the lowering of blood cholesterol, insulin, glucose and corticosterone levels (131). Data from the AIBL study has shown that higher levels of PA are associated with higher HDL levels, lower insulin and triglyceride levels, as well as a reduced plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio. After stratification by *APOE* ε4 allele carriage, it was evident that only *APOE* ε4 non-carriers received the benefit of reduced plasma $A\beta_{1\text{-}42/1\text{-}40}$ ratio from PA. This analysis was

completed in 546 cognitively normal participants. Additionally, a PiB-PET sub-study on 116 of these participants determined that brain Aβ levels were lower in higher exercising *APOE* ε4 allele carriers (with 116 included in the additional PiB PET sub study; 132). Studies have shown that moderate levels of PA carried out for less than one year may increase the HDL-C levels of young adult women despite only moderate increases in fitness (133). Cross-sectional population-based investigations have indicated that exercise levels attainable by older men and women are also associated with significantly greater HDL-C levels (134). This suggests that frequency of exercise and not high intensity of exercise may be more important in increasing HDL-C levels (135).

Insulin sensitivity has been shown to improve with aerobic exercise, suggesting a potential benefit of improved glucose metabolism on cognitive processes (136). In animal models, exercise-induced cognitive benefits have been linked to improved energy metabolism and insulin signalling in the brain (137). Rat studies of induced diabetes for example, have shown that aerobic exercise can decrease tau phosphorylation and APP expression, and increase levels of some proteins related to the insulin/IGF-1 pathway in the hippocampus (138). The exercise also improved performance of the rats in the Morris water maze test, compared to sedentary controls.

1.8.2. Smoking

Smoking is an established risk factor for many diseases, but its association with AD is controversial. A recent study by Rusanen *et al.* (139) showed that smoking in mid-life increases the risk of dementia among *APOE* ε4 allele carriers but not among *APOE* ε4 allele non-carriers, the participants were first studied in 1977 and were reassessed in 1998. Mouse models have been used to show that smoking increases the severity of amyloidogenesis, neuroinflammation and tau phosphorylation (140). Controversially, a study by Van Duijin and Hofman (141) concluded that there is an inverse association between smoking and AD. The risk of AD decreased with increasing daily number of cigarettes smoked before onset of disease. Recently, MRI studies have shown cigarette smoking in a young-to-middle aged group is associated with smaller brain volumes (142-147), and greater pack years (calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked) is associated with smaller anterior frontal, temporal and cerebellar grey matter volumes

(143, 144) as well as thinner orbitofrontal cortex (145). In 2010, Cataldo *et al.* (148) carried out a review of case control and cohort studies to examine the relationship between smoking and AD: they reported on 43 individual studies after controlling for study design and quality, and found a quarter (11 of the 43) of these studies had tobacco industry affiliated authors, somewhat unsurprisingly three of these 11 studies found a non-significant relative risk of AD associated with smoking and the remaining eight suggested smoking actually protects against AD. Of the non-tobacco industry affiliated studies, 18 had no significance with respect to AD risk and 14 found a significantly increased risk of AD with smoking.

Nicotine has been shown to modulate the neurotoxic effects of Aβ and exert potent neuroprotective effects (149, 150). The presence of nicotine causes an up-regulation and activation of nicotinic acetylcholine receptors, which in turn protect against Aβ cytotoxicity (150). Cholinergic deficits, characterised by reduced levels of acetylcholine nicotinic receptors are found in AD, and it has been shown that AD patients with an *APOE* ε4 allele have fewer nicotinic receptor binding sites and a depletion of choline (105, 151). Nicotine may provide cascading effects via stimulation of the release of a variety of neurotransmitters involved in cognitive function, including acetylcholine, dopamine, norepinephrine, serotonin and glutamate (152). A study by White *et al.* (152) has shown that wearing nicotine patches for four weeks produced a statistically significant improvement in attention but no improvement in memory or overall clinical status, in eight AD patients. Another four week nicotine patch study did not find any differential improvement in short-term memory with nicotine compared to placebo (153). It is possible that a high peak achieved by injection of nicotine is necessary to produce memory improvement in AD patients, as shown by Newhouse *et al.* (154) and Levin and Torry (155). It is of course important to note that nicotine is merely one component of cigarettes, the effects of nicotine vary considerably from those of smoking, which is already accepted to increase the risk of a variety of medical conditions, including several already linked to AD, such as cardiovascular disease and hypertension. Additional studies are needed to resolve whether smoking is a protective or detrimental risk factor for AD.

1.8.3. Diet

Diet plays a major role in the risk and development of neurodegenerative diseases such as AD (156-158) and chronic diseases of the periphery like CVD and diabetes (159-161).

1.8.3.1. Mechanisms of action of diet

There are multiple potential mechanisms underlying the effects of dietary components on AD risk including: modulating oxidative stress, high density lipoprotein, low density lipoprotein and homocysteine levels to name but a few.

1.8.3.1.1. Oxidative stress

AD brains exhibit considerable evidence of reactive-oxygen species (ROS) and reactive nitrogen species-mediated injury. These species, formed during normal metabolic processes, are generally unstable and highly reactive, for this reason their levels are kept low by efficient antioxidant systems. In some circumstances their production can exceed the antioxidant ability to destroy them, and oxidative stress/damage occurs. The brain is particularly vulnerable to oxidative stress due to its high metabolic activity and the presence of relatively few antioxidant enzymes; antioxidant nutrients are a natural defence mechanism against these processes (162). Evidence suggests that oxidative stress contributes to the pathogenesis of AD, therefore increasing the intake of antioxidants could counteract the detrimental effects of oxidative stress and thereby potentially reduce AD risk. Many foods have antioxidant properties including turmeric, green tea, fruit and vegetables and supplements like Ginkgo biloba extracts.

Curcumin, a constituent of the Indian spice turmeric, is a strong antioxidant with anti-tumour and anti-inflammatory properties. The prevalence of AD in populations that are between the ages of 70 and 79 years is reported to be 4.4 fold less in India (where curcumin consumption is high) than in the United States of America (88). While this would imply that the incidence of AD in India is much lower, these figures may be heavily impacted by underreporting, reduced longevity and other health aspects such as obesity in the USA. Despite this, animal work certainly suggests that curcumin may play a role in combating AD. In mouse models of AD, curcumin has previously been shown to decrease levels of oxidised proteins and induce a 43-50% decrease in soluble

Aβ, insoluble Aβ and amyloid plaque formation. Furthermore, curcumin was shown to inhibit both the formation and growth of Aβ fibrils from Aβ in a dose-dependent manner (163). However, a pilot study in AD patients failed to show evidence of a benefit of curcumin therapy (164). There are several reasons as to why this study might have failed; the AD patients may have been late stage and therefore exhibiting irreversible pathology. Also, the uptake of curcumin in the gut can be poor which may account for the lack of clinically significant results. To date no clinical study has measured curcumin levels in the blood to investigate bioavailability. Consumption of curcumin together with black pepper increases uptake, the component within the black pepper that facilitates this uptake is piperine. Piperine also exhibits antioxidant activity with significant chemo-preventative and immunomodulary effects (164). However, treating an older population with a combination of piperine and curcumin is not recommended as piperine is known to irritate the bowel, which could be a serious complication. Studies are being conducted with the aim of improving the bioavailability of curcumin for future research (165).

Studies in India and Singapore have shown an association between green tea consumption and decreased risk of AD; the most active ingredient of green tea is epigallocatechin gallate (EGCG), and consumption of at least five cups per day is required for effects to be observed (166). EGCG is a polyphenol which exhibits potent antioxidant and anti-inflammatory properties, and it has been shown to confer neuroprotection in AD mouse models. The accumulation of iron at sites where neurons degenerate is thought to play a major role in the oxidative stress-induced process of neurodegeneration. EGCG possesses iron-chelating properties, thereby conferring additional potential protective mechanisms (88). EGCG is also known to activate α-secretase which cleaves APP via the non-amyloidogenic pathway (Figure 1.2) to prevent Aβ formation (166).

A high intake of vitamin C and vitamin E from food may be associated with a lower incidence of AD. Studies on supplement use are, however, prone to bias; a select proportion of the population take supplements and these people may also either possess more health problems or demonstrate additional beneficial lifestyle behaviours. There are also differences in absorption and biological activity between antioxidants from foods and antioxidants from supplements. Several studies have examined the relationship between AD and intake of vitamin C and E from supplements: a case

control study (167) and a prospective study (168) showed no association, whilst another prospective study showed association with vitamin C but not with vitamin E (169). A randomised controlled trial performed on AD patients, showed that those who took vitamin E supplements had a slower progression of the disease than those who ingested a placebo; it is conceivable therefore that high intake of antioxidants may slow the progress of AD by reducing neuronal loss due to oxidative damage (170). Supplement intake is however of shorter duration than food intake which generally reflects long-term ingestion. If duration of antioxidant intake is more important than dose, constant life-time intake from food would be more likely to be related to AD risk than short-term high intake from supplements (162).

The standardised extract from the leaves of the Ginkgo biloba tree, labelled EGb761 (Ginkgo biloba) is one of the most popular herbal supplements. The proposed action of Ginkgo biloba relates to its free-radical scavenging action, which is supported by numerous *in vitro* and *in vivo* studies (171), including one in which pre-treating cerebellar granule cells with Ginkgo biloba effectively attenuated oxidative damage (172). Abnormalities in mitochondrial function are suggested to be associated with the pathological changes seen in AD, and Ginkgo biloba has been proposed to have direct protective effects on mitochondria (173): this may also contribute to its antioxidant effects, as the mitochondrial respiratory chain is both the major target and the major source of ROS. Ginkgo biloba may also stabilise the cellular redox state by up-regulation of the protein level and activity of antioxidants (174). Reports suggest that despite the preclinical promise that Ginkgo biloba may be effective in the treatment and prevention of AD, this has not completely translated to clinical research benefits. Most reviews to date have shown inconsistent results and failed to draw conclusions as to whether Ginkgo biloba has clinically-relevant benefits in AD patients. One study with 176 participants could not demonstrate any evidence of effectiveness of 120 mg of Ginkgo biloba in mild to moderate dementia (175). Furthermore, the Ginkgo Evaluation of Memory Study found no favourable effects of 240 mg Ginkgo biloba on the prevention of dementia onset in older people without AD or with MCI (176). In a review by Weinmann *et al.* (177) however, it was concluded that Ginkgo biloba ingestion produced favourable effects compared to placebo in relation to improvements in cognition for patients with AD, vascular or mixed dementia. Their analysis showed that a dosage of 240 mg might be necessary to yield results. Participant numbers ranged

from 18 participants to 513, the average number of participants over the nine studies was 249. All studies involved ingestion of either 120 mg or 240 mg Ginkgo biloba or placebo, with the duration of the studies ranging from 12 to 52 weeks.

1.8.3.1.2. Fats and cholesterol

HDL particles carry cholesterol to the liver where it is processed and excreted from the body, whereas LDL particles adhere to the walls of arteries causing plaque build-up, which decreases blood flow and increases risk of a cardiac event. Therefore HDL cholesterol (HDL-C) has been termed 'good cholesterol' and LDL cholesterol (LDL-C) 'bad cholesterol'. Moderate alcohol intake has been associated with protection against CVD in observational studies, an effect that appears to be mediated in large part by alcohol-induced increases in HDL-C concentrations. The increase in HDL-C concentrations with moderate alcohol intake results from increased transport rate of the major HDL apolipoproteins apoA-I and -II (178).

In some European studies, a moderate dietary intake of alcohol is associated with a decreased risk of dementia; however, the specific alcoholic beverages that mediate this benefit are unknown, and evidence to the contrary also exists. A study by Luchsinger *et al.* (179) concluded that individuals older than 65 years of age had a lower risk of AD if they demonstrated a light to moderate intake of wine, there was no association with beer or liquor, and this was restricted to individuals without the *APOE* ε4 allele. One possible explanation for this could be that the presence of the *APOE* ε4 allele increases the risk of AD in such a way that it overwhelms the potential protective mechanism of wine. While it is not known why *APOE* ε4 allele carriers do not benefit from moderate alcohol consumption, it is known that the *APOE* ε4 allele is associated with poor clearance of Aβ and that alcohol increases HDL which is thought to play a role in Aβ clearance. It is feasible therefore that the influence of moderate alcohol intake is not sufficient for *APOE* ε4 allele carriers to increase HDL levels and thus increase clearance of Aβ. We cannot exclude the possibility that it is not the effect of alcohol on HDL, rather that other social and lifestyle related factors that 'go together' with certain drinking habits are responsible for the reported favourable associations between alcohol drinking and cognition. It is worthy of note however that there are also limitations to previous studies on alcohol and risk of AD, including misclassification of alcohol

consumption and assumption that alcohol intake was stable over time, when it is probable that there is significant intra-person variation in alcohol intake.

If alcohol does provide a true protective effect, it is possible that the risk of dementia is decreased at the expense of increasing other co-morbidities, including factors associated with the high caloric intake of alcohol. Many of these alcohol-related studies have not taken into account nutritional status of participants, for example whether they have other risk factors like obesity or fatty liver, as under these conditions it is easily appreciable that alcohol would not show a positive effect. Furthermore, the proposed beneficial effect of alcohol on AD may be due to its association with decreased risk of coronary disease, a risk factor for AD (179).

High intakes of total fat, saturated fat and cholesterol are associated with increased risk of AD and CVD. High intakes of polyunsaturated fatty acids (PUFA) are associated with a decreased risk for AD. There are two types of PUFA associated with AD, omega-3 PUFA and omega-6 PUFA. One example of an omega-3 PUFA dietary source is fish, and consumption of fish has been shown to be inversely related to AD, even at relatively low levels. The omega-3 PUFA in fish have anti-inflammatory properties, they can decrease the production of cytokines in humans; these are immune signalling molecules believed to be involved in the pathogenesis of AD. PUFA also have a role in brain development and the functioning of nerve membranes, and so may be of importance in the regeneration of nerve cells. Brain autopsies have shown decreased PUFA content in the hippocampus and frontal grey matter of AD brains, compared to normal aged brains (180).

Docosahexaenoic acid (DHA) is an omega-3 PUFA, and fish is a direct dietary source of DHA. DHA can also be synthesised endogenously through a process of desaturation and elongation of precursor omega-3 PUFA, alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA). DHA is an important lipid in neuronal membranes, where it has a structural and functional role. DHA modulates properties of the hydrophobic core of the membrane bilayer, including conferring a high degree of flexibility, and directly interacting with membrane proteins, thereby impacting on speed of signal transduction, neurotransmission, and the formation of lipid rafts (181). Animals fed diets enriched with omega-3 PUFA have better regulation of neuronal membrane excitability, increased levels of neurotransmitters, higher density of neurotransmitter

membrane receptors, increased hippocampal nerve growth, greater fluidity of synaptic membranes, higher levels of antioxidant enzymes, decreased levels of lipid peroxides, reduced ischemic damage to neurons, increased cerebral blood flow and superior learning acquisition and memory performance, compared to animals fed control diets (182). Other findings reported by Morris *et al.* (183) showed that people who consumed one fish meal a week had a 60% decreased risk of developing AD than those that rarely or never ate fish. Of the omega-3 PUFA only DHA was protective against development of AD. Intake of ALA was protective but only among people with the *APOE* ε4 allele. No protective effect was associated with EPA consumption; however, intake was only at low levels, so an effect at higher intake levels cannot be ruled out.

High intakes of monounsaturated fatty acids (MUFA) are also associated with lowering LDL cholesterol and decreasing risk of AD (184), primary sources of MUFA include olive oil and canola oil. It has been recommended that people at risk of obesity, metabolic syndrome and diabetes should avoid refined sugars and white flour, and should eat whole unrefined foods, with natural fats, especially fish, nuts, olives and olive oil, in order to reduce their risk of AD in later life (88). Studies of olive oil have shown that the MUFA are not the only beneficial components of the oil though, as (virgin) olive oil contains biophenols and squalenes (oleocanthal, tyrosol, hydrotyrosol, and oleuropein) which provide exceptional antioxidant activity; for example, biophenols suppress the synthesis of LDL (185).

1.8.3.1.3. Homocysteine level

Elevated plasma homocysteine levels have been associated with increased risk of carotid atherosclerosis and stroke (186). Both carotid atherosclerosis and stroke have in turn been shown to increase the risk of AD (187). It can subsequently be hypothesised that high homocysteine levels are a risk factor for AD. Numerous studies have investigated this association, and reported a strong link between high homocysteine levels and increased risk of AD (188-195). Furthermore, high levels of homocysteine at AD diagnosis have been associated with rapid disease progression, increased cognitive decline and atrophy of the medial temporal lobe (188, 196-199). Folate, vitamin B_{12} and B_6 are co-factors in the methylation of homocysteine (200), which is an intermediate compound of the metabolic cycle of methionine (201). When the essential amino acid

methionine provides a methyl group to other cell compounds, homocysteine is formed. The cycle is completed by remethylation of homocysteine to produce methionine, a step that requires both folate and vitamin B_{12}. Another pathway for removal of homocysteine is catabolism and entry into the Krebs cycle, which requires vitamin B_6. Blockage of either pathway increases homocysteine concentrations (202). Blood levels of folate, vitamin B_{12} and vitamin B_6 have been inversely related to homocysteine levels (203-207), and therefore anyone with a nutritional deficiency that leads to low blood concentrations of folate, vitamin B_{12} or vitamin B_6 is at increased risk of raised plasma levels of homocysteine (208). Several clinical trials have shown that plasma homocysteine level can be decreased with increased intakes of fruit and vegetables, as these are high in folate (209-211). Fung *et al*. (212) reported that their western diet pattern was positively associated with homocysteine concentration, and their prudent diet pattern (explained further in section 1.8.3.2.2) was inversely associated with homocysteine level, and Gao *et al.* (213) found those with high adherence to a refined cereals pattern were at greatest risk for high homocysteine level and low vitamin B status compared to those with a high adherence to a fruit and milk pattern in a Chinese population. Fruits and vegetables have a pivotal role in the Mediterranean diet (MeDi, see section 1.8.3.2.1), and homocysteine levels have been shown to be lower in those in the highest MeDi adherence tertile which may in part explain the reported associations between high MeDi adherence and lower AD risk (2-4, 214).

Folate occurs in a variety of foods, especially dark green leafy vegetables, citrus fruits, legumes, egg yolks and liver and kidney. Some bread, cereals and pasta are fortified with folic acid; the supplementation form of naturally occurring folate. There is greater folate concentration in vegetables than fruit, which may explain the findings of an inverse association between vegetable intake and cognitive decline but no association with fruit intake (215). The human body needs folate to synthesise DNA, repair DNA, and methylate DNA. Low levels of folate can lead to homocysteine accumulation which increases AD risk. By keeping homocysteine levels low, folate can protect cerebral vessels and can prevent accumulation of DNA damage in neurons caused by oxidative stress and facilitated by homocysteine.

1.8.3.2. Dietary patterns

Diet is one of the few modifiable preventive health risk factors that affects all people and is under the control of the individual. It is clear that diet can influence the levels of undesirable substances in the body, as detailed in the sections above on LDL and homocysteine. There is also increasing evidence to suggest that components in the foods we consume interact with each other to impart disease protection and a higher level of health; this is referred to as food synergy (216, 217). The evidence for health benefits appear stronger when foods are inserted into synergistic dietary patterns rather than considered as individual foods or food constituents (218). As an alternative approach, dietary pattern analysis has emerged (219). Identified dietary patterns are specific to the populations analysed and may not usually be generalised to all populations. Future research needs to be conducted within different racial and ethnic groups with varying dietary practises (220).

1.8.3.2.1. Mediterranean diet

The Mediterranean diet (MeDi) is not a specific diet but a combination of eating habits traditionally followed by people in the countries bordering the Mediterranean Sea (201). The MeDi is characterised by a high intake of vegetables, legumes, fruits, cereals, fish and unsaturated fatty acids (mostly in the form of olive oil), low intake of saturated fatty acids, meat and poultry, low-to-moderate intake of dairy products (mostly cheese and yoghurt), and a regular but moderate amount of alcohol (mostly wine and generally with meals). The MeDi includes many dietary components reported to be beneficial in reducing neuronal degenerative disease risk (2-4, 221-225), and therefore has received much attention.

1.8.3.2.1.1. Mediterranean diet, cognition and Alzheimer's disease risk

Scarmeas *et al*. have published several papers on MeDi and AD in an American population (2-4). In these studies they have found that higher adherence to the MeDi is associated with lower risk for AD and slower cognitive decline. In 2009, they reported that higher adherence to the MeDi was associated with lower risk of developing MCI and MCI conversion to AD (222). Compared to subjects in the lowest MeDi adherence tertile, subjects in the middle MeDi adherence tertile had 17% less risk of developing MCI, while those in the highest MeDi adherence tertile had 28% less risk

of developing MCI. There were 482 subjects with MCI, 106 of whom developed AD during 4.3 years of follow-up. Those in the middle tertile had a 45% less risk of developing AD and those in the highest tertile had a 48% less risk of developing AD. The associations between MeDi and risk for development of MCI and of MCI conversion to AD did not attenuate, even when simultaneously adjusted for more commonly considered potential confounders such as age, gender, ethnicity, education, *APOE* genotype, caloric intake and body mass index (BMI). In a French study of 1410 individuals aged 65 and over, without dementia at baseline and with a follow-up assessment within five years, it was observed that each additional unit of the MeDi score was associated with fewer Mini-Mental State Examination (MMSE) errors at follow-up (1).

By contrast, the Personality and Total Health (PATH) through life study observed that greater adherence to MeDi was not protective against cognitive decline. The apparent lack of protection could be explained by the heterogeneous nature of the study population. Furthermore, only 66 participants from the original 1528 demonstrated any cognitive decline in the four year follow-up; a limitation with respect to sufficient statistical power to detect the MeDi effects. The primary outcomes of the study were MCI, a clinical dementia rating of 0.5, or any mild cognitive disorder at follow-up (226).

MeDi adherence is also proposed to affect subsequent AD course. In another study by Scarmeas *et al.* (4) it was shown that higher adherence to the MeDi was associated with lower mortality in AD (Figure 1.7). The authors noted a gradual reduction in mortality risk for higher MeDi adherence tertiles, which suggests a possible dose-response effect. Translating results into mean survival times, as compared with subjects in the lower MeDi adherence tertile, those in the middle MeDi adherence tertile lived 1.33 years longer, whereas those in the highest MeDi adherence tertile lived 3.91 years longer. Scarmeas *et al.* detected no differences in MeDi adherence between *APOE* ε4 carriers and non-carriers, and no modification of the MeDi-mortality association when adjusted for *APOE* genotype.

A prospective study by Gu *et al.* (227) concluded that a favourable association between higher adherence to MeDi and lower risk of AD did not seem to be mediated by high sensitivity C-reactive protein (CRP; index of systemic inflammation), fasting insulin or

adiponectin (indices of metabolic profile). While biomarker levels were only measured at baseline, it has been shown that circulating levels of these biomarkers are relatively stable over three years (228, 229). Other aspects of the inflammatory and metabolic pathways were not captured by the biomarkers measured by Gu *et al*. and may however, be relevant to the observed MeDi-AD association, for example α1-antichymotrypsin and interleukin 6.

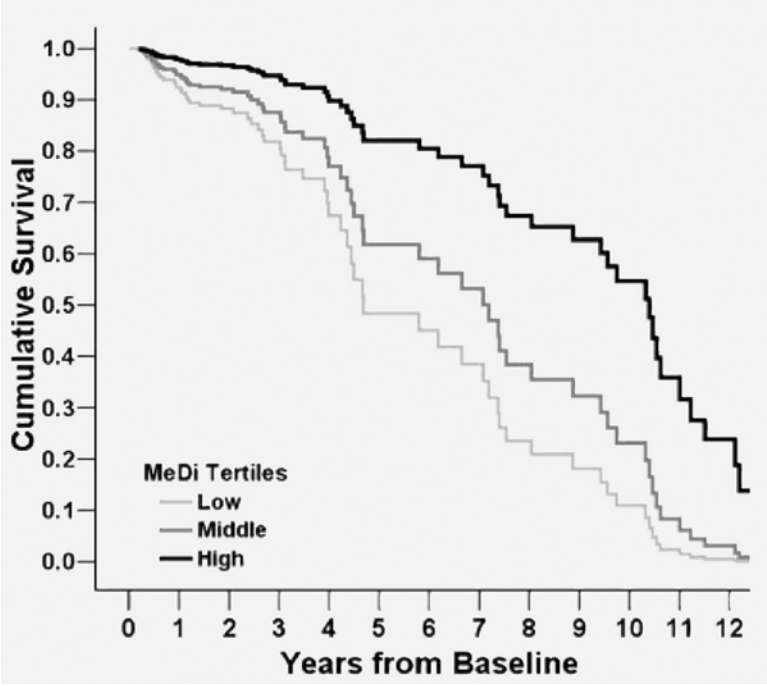

Figure 1.7: *Survival curves based on Cox analyses comparing Alzheimer disease mortality in participants belonging to each Mediterranean diet (MeDi) tertile (p for trend = 0.004). Low tertile (score 0 to 3) corresponds to lower adherence to MeDi, middle tertile (score 4 to 5) to middle adherence, and high tertile (score 6 to 9) to higher adherence to MeDi. The figure is derived from a model that is adjusted for period of recruitment, age, gender, ethnicity, education, APOE genotype, caloric intake, smoking, and body mass index. From Scarmeas et al. (4).*

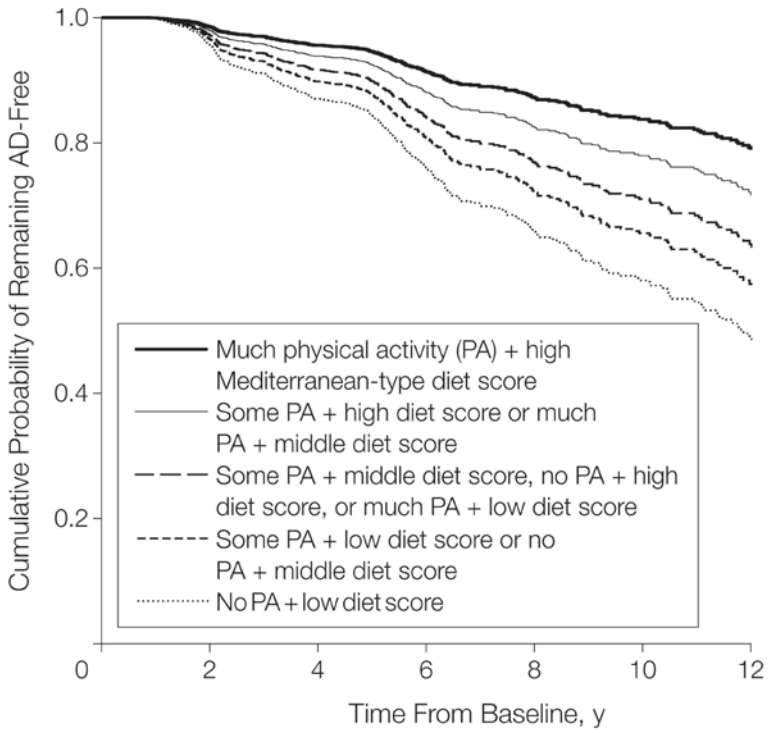

Figure 1.8: *The probability of staying Alzheimer's disease free associated with physical activity (PA) and Mediterranean diet (MeDi) adherence. Adapted from Scarmeas et al. (125).*

Figure 1.8 illustrates the synergistic relationship between diet and PA on AD risk in an American population. People undertaking more PA with higher adherence to the MeDi have a higher probability of staying AD-free for longer, than those who undertake no PA and have a low adherence to the MeDi.

1.8.3.2.1.2. Mediterranean diet and Alzheimer's disease risk factors

The MeDi has been associated with lower risk for dyslipidaemia (214, 230), hypertension (214, 230-232), abnormal glucose metabolism (230, 231), and CVD (223, 230, 233, 234) which are all risk factors for AD.

Scarmeas *et al.* (235) demonstrated an association between MeDi and cerebrovascular disease, specifically infarcts measured by MRI; subjects within the highest MeDi adherence tertile had approximately 40% reduction in the likelihood of a brain infarct (Odds Ratio (OR): 0.64; 95% confidence interval (CI): 0.42 - 0.97; *p* for trend = 0.04). This cohort of 707 elderly individuals was multi-ethnic, including Caucasians, Hispanics, and African American participants, which increases the translational nature

of these results. Hispanics adhered more, and African Americans less to the MeDi, while ethnicity was not related to infarcts.

Gardener *et al*. (236) has shown that MeDi score is inversely associated with risk of the composite outcome of ischemic stroke, myocardial infarction, or vascular death after nine years of follow-up in American males and females, with a mean age of 69 years. Dietary intake was collected at baseline and substantive changes in diet could have preceded the pathological changes that were observed. The number of ischemic strokes and myocardial infarctions was relatively small and therefore may not have provided sufficient power to detect a significant relationship for these outcomes individually. In a secondary CVD prevention trial, Vercambre *et al*. (237) reported that supplements of vitamins E and C, and beta-carotene did not reduce CVD recurrence or influence cognitive decline. In addition in 2504 of these women, who were ≥ 65 years of age and had a follow-up of 5.4 years, adherence to the MeDi was not associated with cardiovascular profile at baseline or with mean annual rate of cognitive change. Prevention of cognitive decline may be more challenging in those with existing vascular disease or risk factors. It is important to note however, that dietary intake was only measured at baseline; this may not reflect long-term dietary patterns, which may be more etiologically relevant.

A significant inverse association between MeDi and both systolic and diastolic blood pressure was observed in a cross-sectional study from a Greek population of 20,343 men and women who had never had a diagnosis of hypertension. It was found that vegetables and fruit were the principal factors that explained the overall effect of the MeDi on blood pressure (232). However the components of fruit and vegetables that confered this benefit are currently unknown.

In a Greek cohort, Trichopoulou *et al*. (223) found that a higher adherence to the MeDi was associated with a reduction in total mortality, with a two-point increment in the MeDi score corresponding to a 25% reduction. Reduction in mortality was evident with respect to deaths resulting from CVD and cancer, but was more pronounced with respect to the former. However, this study has limitations as it had a short follow-up period of 44 months and dietary data was collected only at baseline, therefore, changes in diet over the follow-up period may have indeed influenced these results.

A population of 1507 men and 832 women from two different studies in 11 European countries have shown that higher adherence to the MeDi is associated with a reduced risk for all-cause mortality (Hazard Ratio (HR): 0.77; 95% CI: 0.68 – 0.88), CHD (HR: 0.61; 95% CI: 0.43 – 0.88), CVD (HR: 0.71; 95% CI: 0.58 – 0.88) and cancer (HR: 0.90; 95% CI: 0.70 – 1.17). This study had a follow-up period of 10 years, and in this time, 935 participants died (371 from CVD, 233 from cancer, 122 from CHD and 23 from other causes, and 186 had an unknown cause of death). A MeDi score of four or above was associated with a lower risk of all-cause and cause specific mortality; the strongest observation was observed for CHD (234).

A large study in the USA has found that MeDi adherence is significantly associated with reduced all-cause, CVD and cancer mortality. During ten years of follow-up, 27,799 deaths were recorded, from an initial cohort of 380,296 men and women. In men, the multivariate hazard ratios of mortality from all-cause, cancer, and CVD for the highest versus the lowest level of adherence was 0.77 (95% CI: 0.74 – 0.80), 0.79 (95% CI: 0.73 – 0.87) and 0.76 (95% CI: 0.68 – 0.88), respectively. In women, higher adherence was associated with a 22%, 14% and 21% decreased risk of all-cause ($p < 0.001$), cancer ($p < 0.01$) and CVD ($p < 0.01$) mortality, respectively. The beneficial effect was more pronounced among smokers with high adherence to the MeDi (238). Furthermore, Benetou *et al*. (239) reported a two-point increase in MeDi score was associated with a 12% reduction in cancer incidence (HR: 0.88; 95% CI: 0.80 – 0.95). This was in a Greek cohort of 10,582 men and 15,041 women, of which 421 men and 430 women reported incident cancer cases over a median follow-up of 7.9 years.

Mendez *et al.* (240) have investigated MeDi and obesity. In people already overweight when the study started, 7.9% of women and 6.9% of men became obese over the mean 3.3 year follow-up, and a high MeDi was associated with significantly lower likelihood of becoming obese (women; OR: 0.69; 95% CI: 0.54 – 0.89. men; OR: 0.68; 95% CI: 0.53 – 0.89). However, in normal weight individuals who became overweight, MeDi was not associated with a lower risk for this progression. Although weight was measured at baseline by study co-ordinators, it was self-reported at follow-up; measurement error may have attenuated the results. Mendez *et al.* used an extensive computerised diet-history instrument with over 600 items to capture intake over the previous year. The number of items included is greatly increased compared to other

studies (5, 235, 238, 241, 242), and participant fatigue when completing a questionnaire of this length may have impaired response accuracy.

A higher baseline adherence to the MeDi has been associated with lower cumulative incidence of metabolic syndrome compared to those with the lowest adherence (p = 0.003). These findings were reported in 2,563 participants of the Seguimiento Universidad de Navarra (SUN) Spanish cohort after approximately six years of follow-up. Participants were excluded at baseline if they had a BMI > 30 kg/m^2, or reported risk factors (diabetes, hypertension, hypercholesterolemia, or hypertriglyceridemia), or met the criteria for metabolic syndrome. A limitation of this study is the amount of self-reported information, including waist circumference, blood pressure, high density lipoproteins, triglycerides, and plasma glucose. However, it is worthy to note that more than 45% of the cohort were health professionals, and therefore this self-reported information may be subject to increased bias as a result of health knowledge (242).

In a cohort of 13,380 Spanish graduates, who were followed for approximately four years, those that adhered closely to the MeDi had a lower risk of diabetes. A two-point increase in the MeDi score was associated with a 35% relative reduction in the risk of diabetes (incidence rate ratio 0.65; 95% CI: 0.44 – 0.95). A limitation of this study however, is the number of cases of diabetes identified at follow-up; 33 from an initial cohort of 13,380, which compromises statistical power. Among participants with the highest MeDi adherence, there was a high prevalence of risk factors for diabetes, including older age, higher BMI, family history of diabetes, personal history of hypertension, and a high proportion of ex-smokers. One would therefore have predicted a higher incidence of diabetes amongst this group; the reduced risk of diabetes suggests high MeDi adherence offers substantial potential for diabetes prevention. As reported earlier in this review, the MeDi has a role in CVD prevention, and given that there are many common risk factors for CVD and diabetes, it is expected that the MeDi is also protective against diabetes (241).

When examining factors that influence AD risk, it is important to look at all risk factors that could be associated. For example, whilst there is high adherence to the MeDi in Greece, the rate of AD in Greece is not significantly lower than in other countries where there is a lower MeDi adherence, and this could be due for example to the higher proportion of people that smoke in Greece.

1.8.3.2.2. Other dietary patterns, cognition and Alzheimer's disease

'Healthy' and 'unhealthy' dietary patterns are produced by '*a posteriori'* methods. There are limited studies relating these types of dietary patterns to AD risk in the elderly. This could be due to the identified patterns depending on the study cohort, thereby limiting comparison between studies (243). In a cross-sectional analysis, Samieri *et al*. (6) identified five dietary patterns by cluster analysis, the 'healthy' diet pattern was characterised by higher consumption of fish, fruit and vegetables, and followers had a significantly lower mean number of errors on the MMSE (test of global cognition). In another cross-sectional analysis, Akbaraly *et al*. (7) investigated dietary patterns produced by principal components analysis including a whole foods pattern (rich in fruit, vegetables, dried legume and fish) and a processed foods pattern (rich in processed meat, chocolates, sweet desserts, fried food, refined cereals and high fat dairy products). The authors found that after adjustment for demographic, behavioural and health measures, higher consumption of the whole food pattern was associated with lower odds of cognitive deficit, and higher consumption of the processed food pattern was associated with higher odds of cognitive deficit. However, after adjustment for education, these associations were significantly attenuated. Using reduced rank regression, Gu *et al.* (219) identified a dietary pattern for which higher consumption was strongly associated with lower AD risk. This pattern was characterised by higher intakes of nuts, fish, tomatoes, poultry, cruciferous vegetables, fruits, salad dressing, and dark and green leafy vegetables, and a lower intake of high fat dairy products, red meat, organ meat and butter. Current findings reported in the literature are consistent in their conclusion of a 'healthy' or prudent pattern in opposition to a western, 'unhealthy' or processed dietary pattern (6, 7, 219, 244, 245). The western diet is a dietary habit chosen by many people in developed countries, and increasingly in developing countries. Negative health consequences associated with the western diet are worsened by the lack of portion control coupled with the trademark western sedentary lifestyle. The amount of 'fast food' and 'take-out' restaurants within the Western part of the world is vast. Furthermore, western supermarkets tend to stock far more packaged and processed items than anywhere else in the world (246).

A substantial body of evidence suggests a role for inflammation in AD, the relationship between diet, inflammation and AD, however, remains relatively unexplored. Cavicchia *et al.* (247) have created an inflammatory dietary index which aims to provide a tool

that can categorise individuals' diets on a continuum from maximally anti-inflammatory to maximally pro-inflammatory. The index is relatively novel and has not yet been extensively analysed. Preliminary analysis showed movement toward an anti-inflammatory diet was associated with a decrease in the inflammatory marker serum high-sensitivity CRP (hs-CRP). Analyses using hs-CRP as a dichotomous variable showed that an anti-inflammatory diet was associated with a decrease in the odds of an elevated hs-CRP (p = 0.049). The results are consistent with the ability of the inflammatory index to predict hs-CRP, and they provide additional evidence that diet plays a role in the regulation of inflammation, even after careful control of a wide variety of potential confounders (247).

1.9. Summary

Due to the paucity of information regarding dietary pattern adherence, cognitive decline, risk of AD and AD-related pathology, the results of this thesis will increase current knowledge and potentially significantly impact the AD field. This subject matter has not previously been investigated in such a well-characterised ageing cohort as that of the Australian Imaging, Biomarkers and Lifestyle study of ageing (AIBL). The AIBL study enables access to a wide range of data, including blood and brain imaging biomarkers, a comprehensive neuropsychological assessment as well as lifestyle data. It is hoped that the results of this study will provide guidance regarding aspects of diet that may contribute to reducing risk or delaying the onset of AD.

Chapter Two

Materials and methods

2.1. Introduction

This chapter describes the ethics, research design, participants, and measures of dietary intake, cognitive performance, blood biomarkers, neuroimaging biomarkers, and other materials and protocols that have been used for the work described in this thesis.

2.2. Ethics

The Australian Imaging, Biomarkers and Lifestyle study of ageing (AIBL) is approved by research ethics committees from the major participating institutions; Austin Health, St Vincent's Health, Hollywood Private Hospital and Edith Cowan University. Recognition of the ethics approval from Hollywood Private Hospital was granted by Edith Cowan University to enable me to undertake my PhD research as a student of the university. All participants were provided with an information and consent document that described all procedures, as well as the potential risks and benefits associated with participation. After being provided with documentation describing all procedures, and their risks and benefits, signed informed consent was obtained from each participant before commencement of data collection (for consent and information document see Appendix 1).

2.3. Study population

The AIBL volunteer database (n = 1112) was created by a partnership between leading Australian researchers in Alzheimer's disease and the Australian Commonwealth Scientific Industrial and Research Organisation (CSIRO) in 2005 (11). The aims of this collaboration were to establish a well-characterised cohort of older Australian adults aged 60 years and over, and to collect comprehensive information on cognitive function, amyloid brain load, blood biomarker levels and lifestyle behaviours every 18 months. The AIBL cohort is composed of three main groups:

- cognitively healthy controls (HC)
- individuals with mild cognitive impairment (MCI)
- individuals with early-stage Alzheimer's disease (AD).

A number of high impact studies investigating cognitive function in the aged, lifestyle, neuroimaging and blood biomarkers of AD have been published utilising data from the AIBL study (for exhaustive AIBL publication list visit www.aibl.csiro.au). Figure 2.1 shows the tracking of the AIBL cohort from baseline to 36 month follow-up.

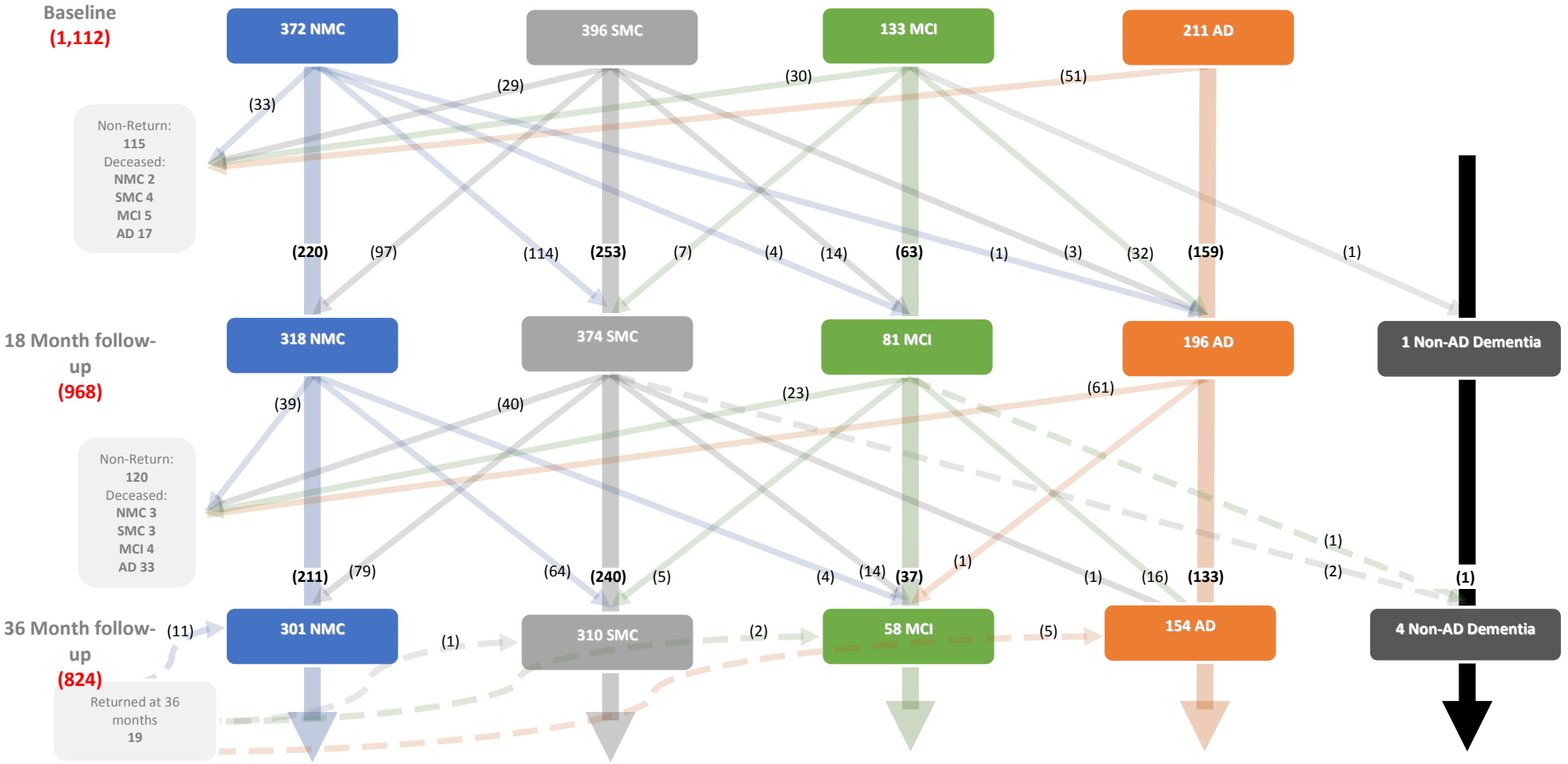

Figure 2.1: *Detailed tracking of the whole AIBL cohort between baseline and 36 month follow-up.*

Abbreviations: AD, Alzheimer's disease; AIBL, Australian Imaging, biomarkers and Lifestyle study; MCI, mild cognitive impairment; NMC, healthy control non-memory complainer; SMC, healthy control subjective memory complainer.

2.4. Recruitment, eligibility screening and exclusion criteria

The recruitment of volunteers was conducted through media advertisements (i.e. television and radio commercials and newspaper advertisements) in Melbourne and Perth, Australia, to which more than 4000 individuals responded. Volunteers underwent eligibility screening by telephone, at which time basic demographics (contact details, sex, and age) were obtained. In addition, information regarding medical history was obtained, with specific information collected regarding eligibility. Furthermore, information related to alcohol intake was collected. All AIBL volunteers were aged 60 years and above at baseline, and excluded if they had a history of non-AD dementia, schizophrenia, bipolar disorder, significant current depression, Parkinson's disease, cancer (other than basal cell skin carcinoma) within the last two years, symptomatic stroke, insulin-dependent diabetes, uncontrolled diabetes mellitus or current regular alcohol use exceeding two standard drinks per day for women or four per day for men.

2.5. Classification of participants

All available information (medical history, memory complaints, information from family, and neuropsychological test data) was utilised to classify individuals as AD, MCI or cognitively healthy. During clinical review panels, a consensus diagnosis was assigned for each participant, which included consideration of diagnostic criteria, DSM-IV diagnosis (248) and ICD-10 diagnosis (249). Where appropriate, ICD-10 dementia severity rating (249), NINCDS-ADRDA AD diagnosis (probable or possible) and MCI classifications were applied. The clinical review panel comprised old age psychiatrists, a neurologist, a geriatrician and neuropsychologists. MCI diagnoses were made according to the protocol based upon the criteria of Winblad *et al*. (250) which are informed by the criteria of Petersen *et al*. (251). Consistent with Winblad criteria, all participants classified with MCI had either personally, or through an informant, reported memory difficulties (250).

2.6. Study visit

All participants that met the inclusion criteria had a study visit arranged. Study visits were conducted at three locations in Melbourne and one location in Perth. On the morning of the participants' visit, measurements of pulse, blood pressure, height, weight and abdominal circumference were obtained. Following these measurements, an 80 mL fasting blood sample was drawn from the antecubital vein using standard venipuncture

technique. Immediately after blood collection, participants were provided with a light breakfast after which they were required to fill out a medical history questionnaire before undergoing a series of cognitive and mood assessments. All procedures were repeated at 18 month intervals. At baseline and the 36 month follow-up, participants completed the Cancer Council of Victoria food frequency questionnaire (CCVFFQ). At the 36 month follow-up, Perth participants were also given instructions on how to complete the online Australian Commonwealth Scientific Industrial and Research Organisation food frequency questionnaire (CSIROFFQ). At the 72 month follow-up, a subgroup of Perth participants completed a modified CSIROFFQ (further described in section 2.9.3.).

2.7. Measures, materials and instruments

The assessment of each participant required the use of various measures. The measures and methods used for this thesis are discussed in the following categories; 1) Cohort characteristics, 2) Dietary intake assessment, 3) Neuropsychological assessment, 4) Blood biomarker analysis, and 5) Neuroimaging biomarker analysis.

2.8. Cohort characteristics

2.8.1. Demographics, medical history and medication use

All participating individuals completed a detailed questionnaire regarding demographic information, medical history, medication use, as well as specific questions about tobacco and alcohol consumption. Demographic information included: marital status, occupation (past/present), country of birth, living arrangements, handedness, primary language spoken, years of education, primary source of income, participation in community organisations and pet ownership. Current or past history of the following conditions or diseases was collected; hypertension, angina, heart attack, stroke, diabetes, visual defects, visual colour deficiency, cancer, history of falls, thyroid/parathyroid disease, gastric complaints, arthritis, kidney disease, liver disease, joint replacement, epilepsy, serious head injury, neurological disorders, depression, anxiety and psychiatric disorders. The name, dose and frequency of all medications being taken at time of assessment were also recorded on this form.

2.8.2. Blood pressure and body mass index

Blood pressure was measured in the sitting position, before the commencement of neuropsychological assessment. Both systolic and diastolic blood pressures were measured using the *Welch Allyn DS-65 Tycos Trigger*® blood pressure monitor with the *Welch Allyn* blood pressure cuff. Height (cm) was measured using a wall mounted height rod, and weight (kg) using standard scales. Body mass index (BMI) was calculated as weight (kilograms) divided by height (metres) squared (weight/height^2).

2.8.3. Physical activity assessment using the International Physical Activity Questionnaire

Self-reported habitual physical activity levels were measured using the International Physical Activity Questionnaire (IPAQ; 252). The IPAQ is a subjective measure that asks participants to recall the physical activity they completed in the previous seven days. The questionnaire is separated into five different sections and consists of work activity, transportation activity, housework, leisure time activity and time spent sitting. Further, the intensity (i.e. light, moderate, or hard) of the physical activity was quantified. Each question was allocated a metabolic equivalent score (MET); walking was allocated 3.3 METs, moderate activities such as swimming and moderate garden work were allocated 4 METs, whilst vigorous activities were given the metabolic equivalent scores that ranged from 5.5 – 8 METs, depending on the type of activity undertaken (252). The MET scores were then multiplied by the number of minutes per week spent participating in that activity to produce a mean seven day activity score (MET·min/wk^{-1}). This approach was completed as per the IPAQ user manual (http://www.ipaq.ki.se) and previous literature (252, 253). The IPAQ has been validated in various studies which concluded that the IPAQ has acceptable properties for the measurement of physical activity (252, 254). Individuals who did not properly complete the IPAQ, or showed signs of over-estimation or over-reporting (more than two standard deviations above the mean), were excluded from the analyses. The MET·min/wk^{-1} IPAQ score was used to stratify the cohort into tertiles for further analyses.

2.9. Dietary intake assessment

2.9.1. Cancer Council of Victoria food frequency questionnaire

At baseline and the 36 month follow-up, participants completed the CCVFFQ. Food frequency questionnaires (FFQ) have been developed as a means of assessing intakes of foods and nutrients in large scale epidemiological studies and often record intake over the previous 12 months. The CCVFFQ is a modification of an FFQ that was developed in the late 1980s to measure diet in the Melbourne Collaborative Cohort Study, and has been designed specifically for Australian adults. The CCVFFQ is a 74-item self-administered FFQ which can be scanned to facilitate analysis of dietary intake. This FFQ has been validated relative to seven-day weighed food records in pre-menopausal women. Participants were asked to indicate how often they eat each of the 74 food items using 10 frequency response options ranging from 'Never' to '3 or more times per day'. The FFQ also contains three photographs of scaled portions for four foods (used to calculate a portion size calibrator), questions on the overall frequency of consumption of fruits and vegetables (used to calibrate the overestimation of these foods in the food list) and questions on the consumption of foods such as bread that do not easily fit into the frequency format. The 74 food items are grouped into four categories: 1) cereal foods; sweets and snacks: 2) dairy products, meats and fish: 3) fruit: and 4) vegetables, as well as a separate set of questions covering intake of alcoholic beverages. The responses were analysed at the Cancer Council in Carlton, Victoria, to calculate usual daily nutrient intake and grams consumed, using software based on the Nutrient Tables for use in Australia 1995 (NUTTAB95). The output of this analysis consists of the grams of 68 nutrients consumed per day and grams of 101 foods and beverages consumed per day for each participant.

2.9.2. Commonwealth Scientific Industrial and Research Organisation food frequency questionnaire

At the 36 month follow-up, participants in the Perth cohort completed the online CSIROFFQ to add an extra layer of data to that obtained from the CCVFFQ. The CSIROFFQ quantifies intake of over 200 foods and beverages. The online CSIROFFQ was administered at the participant's residence, over the phone or at our research institution. The online delivery of the questionnaire ensures completion of all questions as participants cannot proceed without finishing each section; questions on paper questionnaires by contrast, can easily be missed. Participants were able to complete the

questionnaire in multiple sessions in order to prevent non-completion of questionnaires due to fatigue. Questionnaires were analysed as each FFQ was completed, thus allowing manual coding to be minimal, and costs to be reduced. A paper version of this online CSIROFFQ has previously been validated (255-258); the paper version uses an earlier version of NUTTAB (NUTTAB 1995) compared to the online version (NUTTAB 2006).

Table 2.1 shows an example of the results each participant received immediately after completion of the online questionnaire. The output analysis provided quantitation of grams of 456 foods and beverages consumed per day for each participant. Participant responses were analysed by the CSIRO using food composition derived from the Australian Food and Nutrient Database 2007 (AUSNUT 2007) and the British Food Composition Tables. AUSNUT 2007 contains analytical data published in Nutrient Tables for use in Australia 2006 (NUTTAB 2006) and data from the Food Standards Australia New Zealand 2006 (FSANZ 2006) analytical program, as well as nutrient data borrowed from overseas food composition tables, food label information, imputed data from similar foods and data calculated using a recipe approach.

Table 2.1: *Example of CSIROFFQ results.*

Average daily intake of each nutrient

Nutrient	Average Daily Intake	Nutrient	Average Daily Intake
Water	2959.80 g	Alcohol	19.50 g
Energy (total)	5782.03 kJ	Fat (total)	37.23 g
Energy (excluding dietary fibre)	5460.31 kJ	Saturated fatty acids	11.19 g
Protein	79.50 g	Monounsaturated fatty acids	14.95 g
Carbohydrate (total)	126.91 g	Polyunsaturated fatty acids	7.35 g
Sugars (total)	66.98 g	Linoleic acid	5.44 g
Starch	57.34 g	Alpha linolenic acid	1.59 g
Dietary Fibre	39.96 g	Long chain omega 3 fatty acids	239.27 mg
Vitamin A (as retinol equivalents)	749.26 µg	Cholesterol	171.04 mg

Miscellaneous analysis results

Question	Response
Salt added when boiling foods?	No
Salt added to food at the table?	No
Reduced salt canned vegetables usually consumed?	No

Energy intake from macronutrients

Macronutrient	Energy Intake from Macronutrient	Percentage of Total Energy Intake
Protein	1272.07 kJ	22.00 %
Carbohydrate (total)	2157.44 kJ	37.31 %
Alcohol	565.58 kJ	9.78 %
Fat (total)	1377.57 kJ	23.83 %

Abbreviations: CSIROFFQ, Commonwealth Scientific Industrial and Research Organisation food frequency questionnaire; g, grams; kJ, kilojoule; mg, milligrams; µg, micrograms.

2.9.3. Modified Commonwealth Scientific Industrial and Research Organisation food frequency questionnaire

The existing literature suggests that dietary questionnaires need to be specific to the population being analysed, and also disease specific (259). Certain foods assist with prevention of different diseases. We have modified the initial online CSIROFFQ administered at 36 month follow-up to include foods of interest in the field of Alzheimer's disease research. These new questions were selected based on current literature describing major dietary contributors thought to decrease AD risk. Questions we have added or expanded on include capturing information in relation to the following:

- chocolate type
- increased number of oils – e.g. grape seed, coconut oil added
- wine type
- green and white tea
- pomegranates and pomegranate juice
- types of grapes
- types of berries
- kidney, liver, heart
- type of meat used in stew/casserole/curry/goulash
- type of rice
- type of nuts and seeds
- supplements consumed
- herbs and spices consumed

2.9.4. Food frequency questionnaire completion

The HCs in the AIBL cohort completed each FFQ themselves. The MCI participants had cognitive dysfunction which did not significantly restrict the performance of activities of daily living; therefore they were also able to complete the FFQ without assistance. The majority of AD participants in the AIBL study who completed the FFQ were early stage, mild AD; most were living at home with carers, normally a spouse or family member. The AD participants either completed the FFQs with assistance from their carer; alternatively, the carer validated the completed questionnaires afterwards. In

each case, the carer lived with the AD participant, so either prepared the meals for them, or was aware of meals they consumed.

The nature of the CSIROFFQ prohibits incomplete questionnaires; they cannot be submitted online until all questions are answered. By contrast, the CCVFFQs were manually checked following completion and the participant contacted to provide further information if any questions were left unanswered.

Participants were excluded from analyses if their energy intake was less than 500 kCal for women and 800 kCal for men, or greater than 3500 kCal for women and 4000 kCal for men in either of the dietary questionnaires, as proposed by Willett (260).

2.9.5. Validation study

At the 72 month follow-up assessment, 49 HCs from the Perth cohort were included in a validation study of the modified CSIROFFQ. The validation study required a second specific consent form (Appendix 2). The aim of this study was to validate the modified CSIROFFQ relative to four-day weighed food records in a subset of HC participants.

Participants completed an initial modified CSIROFFQ once before completing a consecutive four-day weighed food record (which was started within the next three days). They then completed the questionnaire once again after the four-day weighed food record (within four days of completion) to assess reproducibility. If participants had access to the internet at home they completed the online questionnaire themselves (n = 47). Participants with no internet access at home completed the FFQ over the phone (n = 2) with a researcher entering the answers into the online form.

Before commencing the validation study, participants attended an orientation session. During the orientation session, verbal and written instructions regarding the completion of the four-day weighed food records (Sunday through to Wednesday) were given in detail to the participants by an accredited practising dietician from Edith Cowan University. Digital scales (model 1348, Propert) weighing to the nearest gram, and measuring cups and spoons were provided to each participant, as was a sample of a completed food record. Each participant was requested to record the weight of all food and beverages consumed during the four-day period (either at home or away from home) and to record brand names, methods of food preparation and ingredients of recipes in a specifically designed diary (Appendix 3). In addition, participants were

asked to record the amount of any leftover food at the end of a meal or snack either at home or away from home and to report any dietary supplements consumed. All food records were reviewed face-to-face with the participant upon completion of the four-day period and any unclear or missing information was clarified/completed. Four-day weighed food records were analysed using FoodWorks 7 Professional version 7.0 (Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD) and the AUSNUT 2007 database of Australian Foods, to yield grams per day of foods and beverages consumed. The grams per day of foods and beverages consumed as documented by the weighed food records was compared to the intake reported by completion of the modified CSIROFFQ.

2.9.6. Mediterranean diet score construction

We followed the most commonly described method to construct the Mediterranean diet (MeDi) score (223). An individual was assigned a value of 1 for each; beneficial component (fruits, vegetables, legumes, cereals, and fish) if his/her caloric-adjusted consumption was at or above the gender-specific median; for each detrimental component (meat and dairy products) where caloric-adjusted consumption was below the gender-specific median; and for a ratio of monounsaturated fats to saturated fats at or above the median. Individuals were also assigned a value of 1 for mild to moderate alcohol consumption (> 5 to < 25 grams per day (g/d) for females and > 10 to < 50 g/d for males). The MeDi score was generated for each participant by adding the scores for each category. Thus, the MeDi score could range from 0 to 9, with a higher score indicating a greater MeDi adherence (227). Table 2.2 shows the components of the MeDi.

Table 2.2: *Components of the Mediterranean diet*

Mediterranean diet
High intake of vegetables
High intake of legumes
High intake of fruits
High intake of cereals
High intake of fish
High intake of unsaturated fatty acids
Low intake of saturated fatty acids
Low intake of meat
Low intake of poultry
Low-to-moderate intake of dairy products
Regular but moderate intake of alcohol

2.9.7. Inflammatory dietary index score construction

A group from the University of South Carolina, led by Professor James Hebert, developed an inflammatory dietary index (247, 261), with the aim of providing a tool that could categorise individuals' diets on a continuum from maximally anti-inflammatory to maximally pro-inflammatory. We provided Professor Hebert's team with baseline CCVFFQ data, from which they constructed an inflammatory dietary index score for each AIBL study participant. The inflammatory dietary index incorporates levels of the following dietary constituents: energy, carbohydrates, protein, total fat, fibre, cholesterol, saturated fat, monounsaturated fat, polyunsaturated fat, niacin, thiamine, riboflavin, iron, magnesium, zinc, vitamin A, C and E, folate, beta-carotene, garlic and onion. As consumption of anti-inflammatory foods decreases, the inflammatory dietary index increases. Professor Hebert's team were blinded to all other AIBL data i.e. clinical classification, neuropsychological assessment data, blood and brain biomarker data etc. and all analysis excepting inflammatory dietary index construction was undertaken by AIBL study researchers in Australia. Table 2.3 shows the components of the inflammatory dietary index.

Table 2.3: *Components of the inflammatory dietary index*

Inflammatory dietary index
Niacin
Thiamine
Riboflavin
Iron
Magnesium
Zinc
Vitamin A
Vitamin C
Vitamin E
Folate
Beta-carotene
Garlic
Onion

2.9.8. Western and prudent diet score construction - factor analysis

We first classified 101 food and drink items from the CCVFFQ into 33 predefined food groups (Table 2.4) to minimize within person variations in intakes of individual foods. Where possible, foods were separated into full and reduced fat groups. Individual food items were preserved if they constituted a distinct item on their own. Factor analysis (principle components) was conducted to derive dietary patterns based on the 33 food groups (262). Food groups were entered into the analysis as absolute weight in grams per day. The factors were rotated by a varimax procedure resulting in non-correlated factors, to facilitate interpretability of the factors. In determining the number of factors to retain, we considered; components with an eigenvalue > 1.25, the Scree tests (263), and the interpretability of the factors. Percentage of variance explained by each factor was not used as this largely relies on the total number of variables included in the analysis. We extracted two patterns from the factor analysis; Table 2.5 shows factor loadings for these two patterns. The larger the loading of a food group to the pattern, the greater the contribution of that food group to that pattern. The first major pattern was loaded heavily with red and processed meats, chips, refined grains, potatoes, sweets and condiments. The second major pattern was loaded heavily with vegetables, fruits and

nuts. We labelled the first pattern as the 'western' pattern and the second pattern as the 'prudent' pattern. Labelling of patterns was completed on the basis of our interpretation of the data and did not represent '*a priori*' patterns of intake. The factor score for each pattern was constructed by summing observed intakes of the component food items weighted by factor loadings. These loadings were used to create a western diet score and a prudent diet score for the CCVFFQ data collected at baseline.

Table 2.4: *Food groupings used in the dietary pattern analysis*

Food group	Items from FFQ in food group
Processed meats	Bacon, ham, salami, sausage
Red meats	Beef, veal, lamb, pork
Fish	Fish, fried fish, tinned fish
Poultry	Chicken
Meat pies	Meat pies
Hamburgers	Hamburgers
Eggs	Eggs
Butter	Butter, butter and margarine blends
Margarine	Polyunsaturated margarine, monounsaturated margarine, margarine
Low-fat dairy	Skim milk, reduced-fat milk, yoghurt, low-fat cheese
High-fat dairy	Hard cheese, soft cheese, ricotta or cottage cheese, firm cheese, cream cheese, full-cream milk, ice-cream, flavoured milk drink
Whole grains	Muesli, porridge, wholemeal bread, multi-grain bread, rye bread
Refined grains	High-fibre white bread, white bread, rice, pasta
Other breakfast cereals	All Bran, Bran flakes, Weet Bix, Cornflakes
Pizza	Pizza
Snacks	Crisps, crackers
Chips	Chips
Sweets	Chocolate, cake, sweet biscuits, sugar
Condiments	Jam, Vegemite
Nuts	Nuts, peanut butter
Potatoes	Potatoes
Garlic	Garlic
Other vegetables	Celery, mushrooms, capsicum, beetroot, onion
Legumes	Green beans, peas, other beans, tofu, bean sprouts, soya milk, baked beans
Green leafy vegetables	Spinach, lettuce
Dark-yellow vegetables	Carrots, pumpkin, zucchini, cucumber
Tomatoes	Tomatoes, tomato sauce
Cruciferous vegetables	Broccoli, cauliflower, cabbage
Fruit juice	Fruit juice
Fruit	Avocado, oranges, strawberries, apricots, peaches, mango, apples, pears, bananas, melon, pineapple, tinned fruit
Beer	Light beer, heavy beer
Wine	Red wine, white wine, fortified wines
Spirits	Spirits

Abbreviations: FFQ, food frequency questionnaire.

Table 2.5: *Factor loadings for the two factors (diet patterns) identified from factor analysis.*

Western diet pattern		Prudent diet pattern	
Factor 1	**Factor loadings**	**Factor 2**	**Factor loadings**
Red meats	0.50	Dark yellow vegetables	0.72
Processed meats	0.49	Other vegetables	0.61
Chips	0.49	Green leafy vegetables	0.47
Refined grains	0.48	Fruit	0.41
Poultry	0.43	Cruciferous vegetables	0.39
Condiments	0.42	Nuts	0.30
Potatoes	0.40	Whole grains	0.29
Sweets	0.38	Tomatoes	0.29
Other breakfast cereals	0.35	Fish	0.29
Meat pies	0.32	Low fat dairy	0.26
Margarine	0.31	Potatoes	0.25
High fat dairy	0.30	High fat dairy	-0.25
Dark yellow vegetables	0.30	Poultry	0.22
Fruit juice	0.30	Garlic	0.21
Snacks	0.29	Snacks	0.20
Beer	0.29		
Hamburger	0.24		
Pizza	0.23		
Low fat dairy	-0.21		

2.10. Neuropsychological assessment

A comprehensive battery of neuropsychological tests was administered to all participants by a trained staff member (11). The tests were chosen by senior neuropsychologists and psychiatrists of the AIBL study, and were selected based on international acknowledgement of these psychometric measures and their use in previous literature. Table 2.6 depicts the order, duration and delay for cognitive assessments and Table 2.7 shows the cognitive tests and the domains they each examine, whilst subsequent sections describe each of the tests administered.

Table 2.6: *Order, duration, and delay for cognitive assessments*

Task	Trial	Time to next part of task	Duration (approximately)
Mini-Mental State Examination			10 minutes
Digit span			8 minutes
Logical memory I	Immediate story recall	25 – 35 minutes	3 minutes *
California Verbal Learning Test-II	Retention and immediate recall	20 minutes	12 minutes *
Rey Complex Figure Test	Copy	3 minutes	2 - 10 minutes *
Controlled Oral Word Association Task			3 minutes
Fruit and furniture total and switching			2 minutes
Rey Complex Figure Test	3 minute delayed recall	27 minutes	2 – 10 minutes *
Boston naming test			5 minutes *
Logical memory II	Delayed recall		2 minutes *
California Verbal Learning Test-II	Delayed recall and recognition		7 minutes *
Digit symbol coding			2 minutes
Rey Complex Figure Test	30 minute delayed recall and recognition		5 – 14 minutes *
Clock			2 minutes
The Stroop test			4 minutes
			Total ~ 75 minutes

*Time of task variable based on participant's completion speed.

Table 2.7: *Cognitive tests and the cognitive domains they examine*

Cognitive Test	Cognitive domain
Mini-Mental State Examination	Global cognition, dementia screening tool
Logical memory I and II	Verbal learning and memory
California Verbal Learning Test-II	Verbal learning and memory
Rey Complex Figure Test	Visuospatial functioning and memory
Clock	Visuospatial functioning
Controlled Oral Word Association Task	Language, attention and fluent productivity
Digit span	Attention, processing speed and working memory
Digit symbol coding	Processing speed and attention
The Stroop test	Attention and cognitive flexibility
Boston naming test	Visual naming
Fruit and furniture total and switching	Language, attention, fluent productivity and executive function

2.10.1. Mini-Mental State Examination

The purpose of the Mini-Mental State Examination (MMSE; 264) is to screen for cognitive impairment or dementia, particularly in the elderly population. The items included in the MMSE measure orientation (to both time and place), attention and calculation, language, as well as immediate and delayed recall. A score is given for each correct answer, with a maximum possible score of 30 points.

2.10.2. Digit span

Digit span is a measure of attention and concentration, and is incorporated in the Wechsler Memory Scale (WMS) and the Wechsler Adult Intelligence Scale (WAIS-III; 265). The digit span task comprises two separate trials; digits forward and digits backward. Firstly, in the digits forwards task, a random number sequence consisting of three numbers is read aloud to the participant, who is then asked to repeat the numbers back to the examiner in the same order in which they were read. This is followed by another sequence of the same length. Following correct performance on the previous shorter sequences, sequences with one extra number are read aloud and the process continues until the participant makes an error on both of the two sequences of the same length, or alternatively until they reach the end of the task. In the digits backwards task,

the process is the same as digits forwards except the participant has to repeat the numbers to the examiner backwards. The sum of correct digits forward and digits backward make up the total *digit span* score.

2.10.3. Logical memory

The logical memory test is from the WMS Revised (266), and assesses the ability of individuals to recall a short story. In this task, the examiner reads a short story to the participant and then prompts them to recall as much information from the story as possible *(logical memory I).* Twenty five to thirty five minutes following the first administration of the story, participants are again asked to recall as much information from the story as they can *(logical memory II*). The test is scored based on the number of correct items recalled.

2.10.4. California Verbal Learning Test

California Verbal Learning Test Version 2 (CVLT-II; 267) is a multiple trial learning and recall task which assesses verbal learning and memory. In this task, the examiner begins by reading a 16-item word list, list A (four words belong to each of four semantic categories), and instructs the participant to then say as many items from the list as they can recall, in any order. During the participant recall, the examiner writes down the responses *verbatim*. This list A trial is then repeated a further four times, with the number of correct items over all five trials summed together, to create the *CVLT-II Retention* score. Following the administration of trial 5 of list A, an interference list (list B), containing different items to the previous list, is then read to the participant, who is prompted to recall as many words as they can from this interference list. Following their recall of list B, participants are asked to name as many words as they can recall from list A forming the *CVLT-II Immediate recall* score. After a 20 minute delay, participants are again asked to recall items from list A, with the number of correct items recalled forming the *CVLT-II Delayed recall* score. A new list of items is then read to the participant who is required to answer ‘yes’ if that item was included in list A, and ‘no’ if it was not. In this new list there are items from list A, list B and intrusions. The number from list A correctly identified is the *CVLT-II recognition* score and items falsely thought to be in list A make up the *CVLT-II false positive* score. Using both of these scores, the *CVLT-II recognition discrimination* score is constructed.

2.10.5. Rey Complex Figure Test

The Rey Complex Figure Test (RCFT; 268) is used as a measure of visuospatial functioning and memory. Participants are presented with a complex figure, and instructed to accurately copy it (*RCFT copy)*. Three minutes following the copy, participants are asked to re-draw the figure from memory to produce the *RCFT 3 minute delay* score. Thirty minutes following the initial copy, participants are asked to again re-draw the figure from memory to produce the *RCFT 30 minute delay* score. All three drawings are scored via standardised procedures to produce the test scores.

2.10.6. Controlled Oral Word Association Task

The Controlled Oral Word Association Task **(**COWAT) is a verbal fluency task (269), measuring fluent productivity in the verbal domain. It is part of the Delis-Kaplan Executive Function System (D-KEFS; 269), which provides a standardised assessment of higher-level cognitive functions in children, adolescents and adults between the ages of 8 and 89. In the letter fluency task, participants are asked to name words that begin with a specified letter (F, A, S), in a time limit of one minute. Participants are also given the following rules to follow; 1) the word must not be a proper noun, 2) you cannot add an ending onto a word (suffix) you have already said, e.g. saying *'run'* and then *'running', 'runner', 'runs'*, and 3) words cannot be repeated. The number of eligible words said for each letter are summed, and form the *COWAT* score.

2.10.7. Category fluency (Animals/Boys' names) and fruit and furniture total and switching

This is part of the Delis-Kaplan Executive Function System (D-KEFS; 269) in the same verbal fluency section as COWAT. The verbal fluency test contains two category fluency trials and a single switching condition. The category fluency trials are administered first and require participants to verbally generate as many category exemplars (animals and then boys' names) as possible; participants are given 1 minute for each category. Responses are recorded on paper by the examiner and the score is the total number of correct responses (number of animals and number of boys' names summed together to form one score). Items are counted as correct if they meet the constraints of the condition and are not repetitions. The second part of the test, the switching task, measures the ability to generate words fluently while simultaneously shifting between over learned concepts (category switching). The participant is given 1

minute to alternately name a piece of fruit, and then an item of furniture. To make the *fruit and furniture total* score, participants are given a score for every piece of fruit or furniture they name, regardless of the order; repeated words are not given a point. To make the *fruit and furniture switching* score, participants are given a point for every correct switch between a fruit and a piece of furniture, even if they have repeated either of the items previously.

2.10.8. Digit symbol coding

Digit symbol coding, a task that measures attention, working memory and speed, is from the WAIS-III (265). The administration of the digit symbol coding task requires participants to copy symbols that have been paired with numbers from a legend/key. The number of correct symbols copied in a 120 second time limit is recorded.

2.10.9. Clock

The clock drawing test is used to screen for dementia as well as for visuospatial, constructional and executive difficulties (270). The participant is provided with an unlined sheet of paper and a pencil and asked to draw the face of a clock with all the numbers on it. The participant is told to make the drawing big. After completion of the clock face, they are instructed to set the time to 10 past 11. The clock is scored out of 10 points with criteria for each point. As an example a score of 1 would be given for an irrelevant, uninterpretable figure or no attempt, a score of 5 would be given for perseverative or otherwise inappropriate arrangement of numbers (e.g. numbers indicated by dots) and hands may be represented but do not clearly point at a number. A score of 10 would be given for a normal drawing, numbers and hands in approximately correct positions, hour hand distinctly different from minute hand and at 11 o'clock.

2.10.10. The Stroop test

The Stroop test is a timed measure of attention and cognitive flexibility. Various versions of the Stroop test are available; in this study the Victorian version was used (271). There are three parts to the task; the first part requires participants to name the colour of dots. They are given a card with four lines of six dots of four colours (red, green, yellow and blue). Participants have to read across the rows as quickly as possible reading out the colour of each dot as they go. The time to complete the task and number of errors made is recorded. For the second task, a new card with four rows each with six

words written in four different colours is used. As quickly as possible participants have to read out the colour the word is written in, again time to complete task and number of errors is recorded. The third task, the colours task, is the most difficult of the three Victorian Stroop tasks, and assesses the examinees' ability to suppress usual responses for a less favourable, yet correct, response. In this task, a card is presented with words printed in any of the four colours, however the words are the four aforementioned colours (e.g. the word *'green'* printed in the colour red). Participants are asked to name the colour of the ink of the printed words, not read the actual word. This task requires the participant to inhibit the automatic response of reading the word, for the more difficult, naming of the colour. Time to complete task and number of errors is recorded in seconds.

2.10.11 Boston naming test

The purpose of the Boston naming test is to assess confrontation visual naming ability using black and white drawings of common objects. The original version was published in 1987 and included 85 ink drawings, which was later shortened to 60 items. The version used in the AIBL study is 30 items, including half of these 60 items. Drawings are two-dimensional line drawings of objects ranging in difficulty, beginning with common objects with high word frequency for example "tree" and "pencil" and ranging to lower frequency items such as "trellis" and "palette". Individuals are allowed approximately 20 seconds in which to name each drawing, if the correct word is not produced in that time, a stimulus cue is given (e.g. "used for writing" for "pencil") and an additional 20 seconds allowed for a response. If the name is not produced in that time, a phonemic cue is given (e.g. "pe" for "pencil") and another 20 seconds allowed for the correct response. Correct responses for the spontaneously correct and stimulus-cued correct answers are combined in calculating the Boston naming test score.

2.10.12 Composite scores

The neuropsychological battery assesses six cognitive domains (verbal and visual memory, executive function, language, attention and visuospatial functioning). Composite scores were calculated for each neuropsychological domain by first converting raw scores for each measure to overall sample-based Z scores, then averaging Z scores for the relevant measures to compute a single composite score for that domain. Neuropsychological tests were assigned to one of the six cognitive

domains on the basis of a consensus among neuropsychologists, psychologists and neurologists involved in the AIBL study (Table 2.8 shows neuropsychological tests included in each composite score; 272). We also constructed a global cognitive score for each individual by summing the six cognitive domain scores and dividing by six.

Table 2.8: *Neuropsychological tests included in each composite score.*

Cognitive Domain	Neuropsychological Test	Outcome Measure	Reference
Verbal memory	Logical memory II	Number of details correctly recalled	Wechsler, 1945 (266)
	CVLT-II delayed recall	Number of words correctly recalled	Delis *et al*. 2000 (267)
	CVLT-II recognition discrimination	Discrimination index for recognition of words	Delis *et al.* 2000 (267)
Visual memory	RCFT 3 minute delay	Number of elements correctly recalled	Meyers & Meyers, 1995 (268)
	RCFT 30 minute delay	Number of elements correctly recalled	Meyers & Meyers, 1995 (268)
	RCFT recognition	Number of elements correctly recognised	Meyers & Meyers, 1995 (268)
Executive function	Stroop C/D	Speed of Stroop Colours/Stroop Dots	Strauss, Sherman, & Spreen, 2006 (271)
	Controlled Oral Word Association Task	Number of words produced in 1 minute	Delis *et al*. 2001 (269)
	Fruit and furniture switching	Number of words produced in 1 minute	Delis *et al*. 2001 (269)
Language	Category fluency (Animals/Boys' Names)	Number of words produced in 1 minute	Delis *et al*. 2001 (269)
	Boston naming test	Number of pictures correctly named	Saxton *et al*. 2000 (273)
Attention	Digit span	Total correct trials (forwards and backwards)	Wechsler, 1997 (265)
	Stroop dots time	Speed of trial	Strauss, Sherman, & Spreen, 2006 (271)
	Digit symbol coding	Number of symbols correctly matched	Wechsler, 1997 (265)
Visuospatial functioning	RCFT copy	Number of elements drawn correctly	Meyers & Meyers, 1995 (268)
	Clock	Score out of 10	Libon, Swenson, Barnoski & Sands, 1993 (270)

Abbreviations: CVLT-II, California Verbal Learning Test-II; RCFT, Rey Complex Figure Test; Stroop C/D, Stroop colours/dots.

2.11. Blood biomarker analysis

2.11.1. Blood collection

Blood was collected using the vacuum method of venipuncture from the antecubital vein. Twenty one gauge 0.75 Winged Infusion Sets were used, with the attachment of the multi-adapter to aid in blood collection. Fasting blood samples were collected into serum-separating (for serum isolation) and tubes containing Ethylenediaminetetraacetic acid (EDTA; for plasma isolation) some of which contained prostaglandin E1 where quiescence of platelets was required (+PGE1, 33.3 ng/ml, Sapphire Biosciences, Waterloo, Australia).

2.11.2. Fractionating plasma from whole blood

The tubes containing EDTA+PGE1 were placed on a rocker for 45 to 60 min following blood collection. After 20 minutes on a rocker, 200 μL of whole blood was transferred to a 1.5 mL tube for DNA extraction (see below), and the EDTA+PGE1 tubes were returned to the rocker. These EDTA+PGE1 tubes were then spun at 200 *g* at 20°C for 10 minutes, with no brake, in an Allegra™ X-15R centrifuge (Beckman Coulter, Brea, CA, USA). Following the spin, tubes were removed from the centrifuge, and the platelet rich plasma supernatants were transferred into fresh tubes. The plasma tubes and EDTA+PGE1 tubes with packed cell volume were then spun again, this time at 800 *g* for 15 minutes at 20°C with the brake on before resultant supernatant was transferred to a new tube. Plasma was aliquoted into 250 μL and 500 μL volumes in Cryobank vials (Nalge Nunc International, Rochester, NY, USA). The plasma samples were snap frozen, prior to being transferred to a liquid nitrogen facility for long-term storage.

2.11.3. DNA extraction and Apolipoprotein E genotyping

DNA was extracted from 200 μL of whole blood using the Blood Mini-Kit (Qiagen, Hilden, Germany). Briefly, each 200 μL of whole blood was added to proteinase K and 200 μL of buffer. After pulse vortexing for 15 seconds, the samples were incubated at 56°C for 10 minutes. Following quick centrifugation, 200 μL of 96-100% ethanol was added to the tube. The mixture was then applied to the QIAamp Mini Spin Column and centrifuged at 6000 *g* for 1 minute. The tube containing the filtrate was discarded and the QIAamp Mini Spin Column placed into a clean tube, and 500 μL of buffer was added to the spin column and centrifuged at 6000 *g* for 1 minute, the filtrate was then discarded and the mini spin column placed into a new tube. Following this, 500 μL of

buffer was added to the spin column and centrifuged at 20,000 *g* for 3 minutes. The spin column was then placed into a clean tube with the addition of 200 μL of distilled water. The tube was incubated at room temperature for 1 minute and then centrifuged at 6000 *g* for 1 minute. The extracted DNA was Apolipoprotein E (*APOE)* genotyped via a method previously described (274). Briefly, genomic DNA was amplified with 200 nM primer (*APOE*-F 5' – GCCTACAAATCGGAACTGGA and *APOE*-R 5' – ACCTGCTCCTTCACCTCGT) in one unit of Platinum Taq polymerase (Invitrogen, Carlsbad, CA, USA), 1.5 mM magnesium chloride, and 200 μM deoxyribonucleotide triphosphate with denaturation at 94°C for 7 seconds, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and then 72°C for 30 seconds. Sequencing was completed with BDV3.1 (Applied Biosystems, Foster City, CA, USA) using the *APOE*-F primer on a 3130-xl genetic analyser. From this, the *APOE* genotype for each participant was determined. The size of the fragments determined the genotype with the possible results being: *APOE* ε2/ε2: 2 fragments (91bp, 83bp); *APOE* ε3/ε3: 3 fragments (91bp, 48bp, 35bp); *APOE* ε4/ε4: 3 fragments (72bp, 48bp, 35bp); *APOE* ε2/ε4: 5 fragments (91bp, 83bp, 72bp, 48bp, 35bp); *APOE* ε3/ε4: 4 fragments (91bp, 72bp, 48bp, 35bp) and *APOE* ε2/ε3: 4 fragments (91bp, 83bp, 48bp, 35bp). *APOE* genotyping was undertaken by Dr Simon Laws.

2.11.4. Pathology blood testing

Numerous blood-based analytes were quantified using standard analysis techniques at PathWest Laboratory Medicine, Nedlands, Western Australia and Melbourne Health, Victoria. Normal ranges for each of these analytes are listed below. From serum clotted and EDTA tubes the following analytes were measured: haemoglobin (115 – 160 g/L), red blood cell count (3.8 – 4.8 $x10^{12}$/L), platelet count (150 – 400 $x10^{9}$/L), white cell count (4 – 11 $x10^{9}$/L), neutrophils (2.0 – 7.5 $x10^{9}$/L), lymphocytes (1.2 – 4.0 $x10^{9}$/L), monocytes (0.2 – 1.0 $x10^{9}$/L), eosinophils (0.00 – 0.50 $x10^{9}$/L), basophils (0.0 – 0.2 $x10^{9}$/L), plasma homocysteine (6.0 – 14.0 μmol/L), plasma glucose (3.0 – 5.4 mmol/L), plasma total cholesterol (< 5.5 mmol/L), plasma triglycerides (< 1.7 mmol/L), plasma low density lipoprotein (< 3.0 mmol/L), and plasma high density lipoprotein (> 1.0 mmol/L). Erythrocyte sediment rate (1 – 35 mm/hr) was measured from sodium citrate tubes.

2.11.5. Plasma apolipoprotein E assessment

Plasma apolipoprotein E (apoE) was measured using a commercial kit (apoE4/Pan apoE Enzyme-linked immunosorbent assay (ELISA), MBL co., Ltd., Woburn, USA). This kit measures the amount of total apoE using affinity purified polyclonal antibodies against apoE by sandwich ELISA. Briefly, the plasma samples were thawed and then diluted 1:500 using the Assay Diluent solution provided. Standards were prepared to working concentrations from dilutions of the provided calibrator, which was reconstituted 1:10 with the assay diluents solution. All plasma samples, Controls, and Standards were run in duplicate. Thawed (room temperature) diluted plasma samples or standards were loaded into microwell strips, coated with anti-Human Pan-apoE antibody. The plates were covered and incubated for 60 min at 37°C prior to being washed four times with wash solution. 100 µL of a peroxidase conjugated anti-apoE polyclonal antibody was then added prior to incubating for 60 min at 37°C. The plates were again washed before 100 µL of the peroxidase substrate was added and incubated for a further 30 min at 37°C. An acid solution (100 µL) was then added to each well to terminate the enzyme reaction and stabilize the developed colour. The optical density of each well was measured at 450 nm using a BMG microplate reader. Concentrations of Pan-apoE were determined from a standard curve based on reference standards. The sensitivity of the assay for pan- apoE is 8 ng/ml. The measurement of apoE was undertaken by Dr Veer Gupta.

2.11.6. Rules Based Medicine biomarker panel analysis

Plasma samples were analysed with a 151-analyte multiplex Human DiscoveryMAP® v1.0 panel (Rules Based Medicine, RBM; Austin, TX, USA). All samples below the lower limit of quantification were classed as missing data. From this panel we analysed markers of inflammation (cortisol, C-Reactive protein (CRP), tumour necrosis factor alpha (TNFα), complement component 3 (C3), cluster of differentiation 40 (CD40), CD40 ligand, migration inhibitory factor (MIF), macrophage inflammatory protein 1 alpha (MIP1α), macrophage inflammatory protein 1 beta (MIP1β), tumour necrosis factor receptor II (TNFRII), immunoglobulin M (IgM), interleukin 10, 13, 18, 3, 4, and 8 (IL10, IL13, IL18, IL3, IL4, and IL8) and interleukin 1 receptor antagonist (IL1ra)) and markers of metabolic syndrome (adiponectin, angiotensinogen, glucagon-like peptide-1, glucagon, insulin, leptin and resistin) for associations with each of the dietary patterns constructed.

2.11.7. Lipid profiles

The Lipoprint system (Quantimetrix, Redondo Beach, CA, USA) was used to examine the plasma cholesterol sub fraction profiles of study participants following the kit instructions. Briefly, 25 μL of plasma EDTA was combined with 200 μL of the supplied loading buffer in glass gel tubes and allowed to polymerise for 30 minutes. Samples were then separated in the electrophoretic chamber for one to one and a half hours at 3 mA per tube. Finally, gels were scanned and the band intensity of each sub fraction was obtained using the supplied software. The high density lipoprotein analysis provided values for ten subclasses of high density lipoprotein which were divided into three major groups named high density lipoproteins large (subclasses 1 - 3), high density lipoproteins intermediate (subclasses 4 - 6) and high density lipoproteins small (subclasses 7 - 10). To obtain the low density lipoprotein sub fraction profiles, 25 μL of plasma was mixed with 300 μL of the loading buffer and analysed in the same way as for the high density lipoprotein kit. The low density lipoprotein analysis provided values for very low density lipoproteins, intermediate density lipoproteins (three subclasses A, B and C separately), and low density lipoproteins (seven subclasses grouped into two: subclasses 1 and 2, and subclasses 3 - 7). Lipoprint analysis was undertaken by Mr Steve Pedrini and Dr Eugene Hone.

2.12. Neuroimaging biomarker analysis

2.12.1. Image acquisition

2.12.1.1. Magnetic resonance imaging

To assess brain structure and volume, participants underwent T1 weighted magnetic resonance imaging (MRI) using the ADNI 3-dimensional (3D) Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence and fluid-attenuated inversion recovery (FLAIR) sequence on 1.5T or 3T scanners.

2.12.1.2. Pittsburgh compound-B positron emission tomography

To determine brain amyloid beta load, participants underwent a [^{11}C] Pittsburgh compound-B positron emission tomography (PiB PET) scan at Austin Health Centre for PET, Melbourne or Western Australia PET and cyclotron service, Sir Charles Gairdner Hospital and Oceanic Medical Imaging, Perth. [^{11}C] PiB production was performed

using a one-step [^{11}C] methyl triflate approach. The average radiochemical yield was 30% after a synthesis time of 45 minutes with a purity of > 98% and a specific activity of 30 ± 7.5 GBq/μmol. Each individual received an intravenous injection of ~370 MBq [^{11}C] PiB over one minute. PET images were acquired on a Phillips Allegro^{TM} (Phillips Medical Systems, Eindhoven, The Netherlands) PET camera, 40 - 70 minutes post-PiB injection. A transmission scan was performed for attenuation correction. PET images were reconstructed using a 3D RAMLA algorithm using a voxel size of 2.0 x 2.0 x 2.0 mm^3 (*x y z*). Summed images for the 40 - 70 minute time frame were used.

2.12.2. Image processing

2.12.2.1. Magnetic resonance imaging tissue segmentation

For each individual, the T1 weighted images were classified into grey matter, white matter and cerebrospinal fluid using an implementation of the expectation maximization segmentation algorithm (275). Briefly, using a mix of Gaussian distributions, this algorithm models image intensity histograms. The parameters of the Gaussian mixture are iteratively updated using an expectation maximisation approach. In order to provide initialization and spatial consistency to the expectation maximisation algorithm, an atlas and associated probability maps of grey matter, white matter and cerebrospinal fluid were spatially normalized to each individual scan, first through an affine registration, and then using a diffeomorphic non-rigid registration (276). The regions of interest (ROI; hippocampus, anterior cingulate gyrus, posterior cingulated gyrus, temporal lobes and others) were propagated from the atlas to the T1W to extract the various regional volumes. To improve the robustness of the approach, the process was repeated nine times, with different elderly atlases, resulting in nine segmentations and nine ROIs. The segmentations and the ROIs were voted to generate a consensus grey matter, white matter and cerebrospinal fluid segmentation as well as a consensus ROI for the investigated brain regions. The grey matter segmentation was then used to mask out non grey matter voxels from the ROI. Region of interest volumes were averaged over both hemispheres. The processing of MRIs was performed by Dr Pierrick Bourgeat of CSIRO, Brisbane. All volumetric measures of brain regions were corrected for intracranial volume (ICV) using the following equation:

Adjusted volume = raw volume – b x (ICV – mean ICV); where b is the slope of the regression of an ROI on ICV.

2.12.2.2. Pittsburgh compound-B positron emission tomography image analysis

Acquired [^{11}C] PiB PET images were processed using a semi-automatic ROI method. The semiautomatic method used a preset template of narrow cortical ROIs that were applied to the PiB scan via placement on the individuals co-registered MRI by an operator who was blind to the individuals clinical status. Co-registration of each individual's MRI with the PET images was performed with SPM5 (Statistical Parametric Mapping 5, University College London, UK; 277). The region of interest template was placed on the co-registered MRI and then transferred to the co-registered PET images. Follow-up PiB images were co-registered with the baseline PiB images and the same ROI templates were applied to baseline and follow-up scans. Standardised uptake values (SUV) for PiB were calculated for all brain regions examined and standardised uptake value ratios (SUVR) were generated by dividing all regional SUV by the cerebellar cortex SUV. The cerebrellar cortex was used as a reference region as it is relatively devoid of senile plaques and shows no PiB binding in controls or AD (48, 278). Region of interest measurements were averaged across both hemispheres. Neocortical Aβ burden was expressed as the average SUVR of the area-weighted mean for the following ROIs: frontal (consisting of dorsolateral prefrontal, ventrolateral prefrontal and orbitofrontal regions), superior parietal, lateral temporal, lateral occipital and anterior and posterior cingulate. The image analysis of PiB PET scans was performed by Dr Victor Villemagne and colleagues of Austin Health, Melbourne.

2.13. Statistical analysis

The data contained within this thesis were analysed using various statistical methods that were deemed appropriate for the hypotheses to be investigated. All statistical analyses were performed using IBM SPSS Statistics 19 for Windows Vista (SPSS Inc., Chicago, IL, USA) or R version 2.14.2 (R Foundation for Statistical Computing, Vienna, Austria). Unless otherwise stated, a *p*-value of 0.01 or smaller determined a significant result for all analyses to balance the risk of type I and type II errors, due to the large number of statistical tests performed.

2.13.1. Chapter 3 statistical analysis

Descriptive data analyses were undertaken to provide means, standard deviations and percentages for the entire cohort, and for the cohort following stratification by clinical

classification. For determination of the absence or presence of a relationship between clinical classification groups, independent samples t-tests were performed for continuous data and chisquare ($\chi 2$) for categorical data. A *p*-value of 0.05 or smaller was deemed significant for these analyses as they are exploratory in nature.

Multinomial logistic regression analyses were run to determine the association between the continuous baseline diet scores (independent variable) and baseline participant clinical classification (dependent variable; HC as the reference). The regression model included age, gender, years of education ($\leq$ 12 years, or > 12 years), *APOE* ε4 allele carriage, country of birth (Australia or other), current smoking status, energy intake, BMI and history of stroke, hypertension, heart attack, diabetes and angina as covariates.

For the remaining analyses, only participants classified as baseline healthy controls were utilised. Descriptive data analyses were undertaken to provide means, standard deviations and percentages for the entire healthy control cohort and for the cohort following stratification by gender and *APOE* ε4 allele carriage. For determination of the absence or presence of a relationship between males and females, and *APOE* ε4 allele carriers and *APOE* ε4 allele non-carriers, independent samples t-tests were performed for continuous data and chisquare ($\chi 2$) for categorical data. Bivariate Pearson's correlations were undertaken to evaluate the relationship between the four diet scores at baseline. Multiple linear regression analyses were run to determine the association between the baseline continuous diet scores and cognitive function variables (in healthy controls only). The multiple linear regression analyses measured the association between each diet score (independent variable) and cognitive function (dependent variable) with age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, energy intake, past smoking status, and history of stroke, hypertension, heart attack, diabetes and angina as independent covariates. In a second model without cardiovascular (CVD) risk factors, the same variables were used excluding past smoking status, history of stroke, hypertension, heart attack, diabetes and angina. Analyses were re-run following gender and *APOE* genotype stratification.

A series of repeated measures linear mixed model (LMM) analyses (using maximum likelihood estimation and an unstructured covariance matrix) were conducted to examine the relationship between baseline diet score and time (baseline, 18 and 36 month follow-up) with respect to cognitive change. In the first model (model 1), baseline diet score, time, *APOE* ε4 allele carriage status (presence or absence of ε4

allele; the most common genetic risk factor for AD), BMI, country of birth (Australia or other), years of education (≤ 12 years, or > 12 years), past smoking status, energy intake, history of angina, stroke, hypertension, heart attack, and diabetes were entered as fixed factors; participant as a random factor; age as a covariate; and cognitive composite score as the dependent variable. Model 2 included the same covariates as model 1, but without the CVD risk factors of past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Analyses were repeated following sex and *APOE* genotype stratification of the cohort. The portion of variance explained was estimated by comparing the fixed effect variance with and without that particular term in the LMM.

Finally, a change in clinical classification variable was made by assigning each participant a 0 if they remained a HC from baseline to the 36 month follow-up and a score of 1 if they changed to MCI or AD status. This change in classification variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between change from healthy control clinical classification to MCI or AD status and the four diet scores divided into tertiles; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The same two adjusted models were used as used in the linear mixed models.

2.13.2. Chapter 4 statistical analysis

Descriptive data analyses were undertaken to provide means, standard deviations and percentages for the entire cohort; these analyses were repeated after stratification of the cohort by gender and *APOE* ε4 allele carriage. For determination of the absence or presence of a relationship between males and females, and *APOE* ε4 allele carriers and *APOE* ε4 allele non-carriers, independent samples t-tests were performed for continuous data and chisquare ($\chi 2$) for categorical data.

2.13.2.1. Metabolic syndrome analysis

A metabolic syndrome index was constructed for each individual. We assigned each participant one point for each of the following five components; 1) obesity (BMI ≥ 25); 2) raised BP (systolic ≥ 140 and/or diastolic ≥ 90 and/or a history of hypertension); 3) high triglyceride level (> 2 mmol/L); 4) low high density lipoprotein level (≤ 1.0 mmol/L) and; 5) impaired fasting glucose and/or a history of diabetes (glucose level 6.1 ≥ x ≤ 6.9 mmol/L). The points were summed to produce an index ranging from 0 - 5, with a higher score indicating higher risk of metabolic syndrome.

Multiple linear regression analyses were run to determine the association between the continuous diet scores (independent variable) and this index, as well as the components of the index separately and additional biomarkers of metabolic syndrome measured as part of the AIBL study (dependent variable): angiotensinogen, adiponectin, glucagon-like peptide 1, glucagon, insulin, leptin, resistin, apoE. The multiple linear regression model included age, gender, years of education (≤ 12 years, or > 12 years), *APOE* ε4 allele carriage, country of birth (Australia vs. Other), BMI and energy intake kCal as covariates. For the components of the metabolic syndrome index analysed separately, the models also contained the four other components as covariates.

A metabolic syndrome index was constructed for 36 month follow-up data, using the same method as for the baseline metabolic syndrome index. The 36 month follow-up index was subtracted from the baseline index to give a value for 'change' in the index over 36 months. If the index increased participants were assigned a value of 1, if there was no change in the index participants were assigned a value of 2, and if the index decreased participants were assigned a score of 3.This 'change' in index variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary pattern adherence and change in metabolic syndrome risk. The four diet scores were divided into tertiles for this analysis; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned

to tertile 2 and the highest adherence third assigned to tertile 3. The same adjusted model was used as in the linear regression models earlier in this section. After stratification of the cohort based on gender and *APOE* ε4 allele carriage all of these analyses were re-run.

2.13.2.2. Cardiovascular disease analysis

A CVD index was constructed for each individual. We assigned each participant one point for each of the following nine components; 1) obesity (BMI ≥ 25); 2) raised BP (systolic ≥ 140 and/or diastolic ≥ 90 and/or a history of hypertension); 3) a history of angina; 4) a history of stroke; 5) a history of heart attack; 6) a history of smoking; 7) a history of diabetes; 8) low physical activity (being in the lowest tertile of physical activity as measured by the IPAQ) and; 9) elevated homocysteine level (> 10 μmol/L). The points were summed to produce an index ranging from 0 - 9, with a higher score indicating higher risk of CVD.

Multiple linear regression analyses were run to determine the association between the continuous diet scores (independent variable) and this index, and additionally the components of the index separately (dependent variable). The multiple linear regression model included age, gender, years of education, *APOE* ε4 allele carriage, country of birth and energy intake kCal as covariates. For the components of the CVD index analysed separately, the models also contained the eight other components as covariates.

Additional CVD biomarkers were measured as part of the PathWest analytes at baseline and 36 month follow-up, including cholesterol, triglycerides, high density lipoproteins, and low density lipoproteins. These biomarkers were investigated in the same linear regression analysis as the CVD index, as well as via a longitudinal change in biomarker level, which was determined for these four biomarkers by subtracting the baseline level from the 36 month follow-up level; these 'change scores' were analysed with the baseline diet scores to investigate dietary patterns being predictive of biomarker level change at 36 month follow-up.

Lipid profiles were further analysed at baseline using a Lipoprint system. From this data the three fractions of high density lipoproteins (large, intermediate and small), very low density lipoproteins, intermediate density lipoproteins (three subclasses A, B and C separately), and low density lipoproteins (seven subclasses grouped into two, subclasses

1 and 2, and subclasses 3 – 7) were investigated in the same linear regression analysis as the CVD index to assess dietary pattern associations with lipid profiles.

A CVD index was constructed for 36 month follow-up data, using the same method as for the baseline CVD index. The 36 month follow-up index was subtracted from the baseline index to give a value for 'change' in the index over 36 months. If the index increased participants were assigned a value of 1, if there was no change in the index participants were assigned a value of 2, and if the index decreased participants were assigned a score of 3. This 'change' in index variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary adherence and change in CVD risk. The four diet scores were divided into tertiles for this analysis; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The same adjusted model was used as in the linear regression models earlier in this section. After stratification of the cohort based on gender and *APOE* ε4 allele carriage all analyses was re-run.

2.13.2.3. Inflammation analysis

A range of inflammation biomarkers were analysed via Rules Based Medicine as part of the AIBL study at baseline. We constructed a blood-based inflammation biomarker index using these biomarkers (cortisol, CRP, TNFα, C3, CD40, CD40 ligand, MIF, MIP1α, MIP1β, TNF RII, IgM, IL10, IL13, IL18, IL1ra, IL3, IL4, and IL8). There are no definitive published 'normal range' levels for some of these biomarkers, we assigned a value of one to participants with biomarker levels falling in the top 20% of each biomarker measured (so if an individual had high levels of these biomarkers we would expect them to have higher inflammation), participants where levels of each biomarker fell outside the top 20% were assigned a value of 0. We then added the scores for each biomarker to construct an inflammation index with a score ranging from 0 to 18. We then made a blood-based inflammation biomarker index 2 using pathology test results

measured at baseline (haemoglobin, red blood cell count, erythrocyte sediment rate, platelet count, white cell count, neutrophils, lymphocytes, monocytes, eosinophils and basophils). Again we assigned a value of one to participants with analyte levels falling in the top 20% of each biomarker and a value of 0 to participants where levels fell outside the top 20%. We then added the scores for each biomarker to construct the second inflammation index with a score ranging from 0 to 10. Multiple linear regression analyses assessed both indexes as well as the components of the indexes separately in relation to the four dietary patterns. The diet score was used as the independent variable, biomarker or index level was used as the dependent variable with the inclusion of age, gender, years of education *APOE* ε4 allele carriage, country of birth, energy intake and BMI as covariates.

At 36 month follow-up, the pathology test results were again measured, we made a 36 month follow-up blood-based inflammation biomarker index 2, using the same method as for the baseline blood-based inflammation biomarker index 2. The 36 month follow-up index was subtracted from the baseline index to give a value for 'change' in the index over 36 months. If the index increased participants were assigned a value of 1, if there was no change in the index participants were assigned a value of 2, and if the index decreased participants were assigned a score of 3. This 'change' in index variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary adherence and change in inflammation level. The four diet scores were divided into tertiles for this analysis; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The same adjusted model was used as in the linear regression models earlier in this section.

To analyse chronic inflammation we assessed the relationship between baseline dietary patterns (independent variable) and the 36 month follow-up blood-based inflammation

biomarker index 2 and the components individually (dependent variable) using linear regression analysis.

All analyses were repeated following stratification of the cohort by gender and *APOE* ε4 allele carriage.

2.13.2.4. Neuroimaging biomarker analysis

Multiple linear regression analyses were run to determine the association between the continuous diet scores and neuroimaging biomarkers. An adjusted model measured the association between each of the four diet scores (independent variable) and brain biomarker levels (dependent variable) with the inclusion of age, gender, years of education, *APOE* ε4 allele carriage, country of birth, energy intake, and BMI as covariates. Baseline diet scores were analysed against baseline MRI brain region volumes and PiB PET SUVR (continuous) as a measure of brain amyloid burden. A 'change' in biomarker level was also determined for MRI brain region volumes and continuous PiB PET SUVR by subtracting the baseline level from the 36 month follow-up level. This 'change' score was then analysed with the baseline diet scores to investigate dietary patterns being predictive of imaging biomarker level change at 36 month follow-up. After stratification of the cohort based on gender, *APOE* ε4 allele carriage and PiB PET status (positive or negative) all of these analyses were re-run.

Finally, we analysed PiB PET SUVR as a binary score (0 = PiB PET negative (SUVR < 1.5), 1 = PiB PET positive (SUVR ≥ 1.5)). This binary score (PiB PET negative as the reference) was used in multinomial logistic regression models to calculate odds ratios with the same confounding variables controlled for as in the multinomial logistic regressions earlier in this section. The four diet scores were divided into tertiles for this analysis (method to calculate tertiles is described in section 2.13.2.3.); odds ratios compared the lowest tertile with the highest tertile. There were insufficient numbers of 'converters' from PiB PET negative to PiB PET positive over the 36 month follow-up period to provide sufficient statistical power to observe associations between change in the PiB binary score, therefore this analysis was not undertaken. After stratification of the cohort based on gender and *APOE* ε4 allele carriage multinomial logistic regressions were re-run.

For all statistically significant associations, the portion of variance explained was estimated by comparing the fixed effect variance with and without that particular term in the linear regression.

2.13.3. Chapter 5 statistical analysis

Descriptive data analyses were undertaken to provide means, standard deviations and percentages for the 49 participants that took part in the validation study; these analyses were repeated after stratification of the cohort by gender. For determination of the absence or presence of a relationship between males and females, independent samples t-tests were performed for continuous data and chisquare ($\chi 2$) for categorical data.

For the reliability study, modified CSIROFFQ data was found to be normally distributed by the Kolmogorov-Smirnov test statistic. Pearson's correlation coefficients between the first and second administration of the modified CSIROFFQ were used to compare the daily food and beverage intakes from the two FFQs and evaluate reproducibility. The intra-class correlation coefficient (ICC) was calculated as an indication of test-retest reliability and a measure of the existing within-subject variability. It is the ratio of variability between participants and total variation, meaning that the ICC is the fraction of the variability due to causes other than variability within a participant. Thus, when the ICC is close to 1, this indicates that a single observation suffices, in that if a subsequent observation were taken, it is likely to be similar to the original. For items with a low consumption we constructed groups for these correlations. The groups were made for steamed, baked or grilled fish, oven baked fish, fried fish, canned fish, milk, cordial, flavoured milk, milkshake or thick shake, mayonnaise, salad dressing, gravy, cheese, cream, yoghurt, ice-cream, custard, cereals, popcorn, fats and oils, nuts, seeds, berries, mushrooms, homemade soup, canned soup, packet soup, souvlaki or gyros, chicken or fish burgers, hamburgers, chicken breast, thigh or wings, meat goulash, curry, stew or casserole and kidney and liver.

Pearson's and intra-class correlation coefficients between the first administration of the modified CSIROFFQ and the four-day weighed food records were used to compare the daily food and beverage intakes from the FFQ and weighed food records to evaluate validation of the modified CSIROFFQ.

The validation study revealed there were 12 items added to the modified CSIROFFQ for which intake as determined by completion of the modified CSIROFFQ was

significantly correlated with intake documented by completion of the four-day weighed food record (as determined by Pearson's correlation coefficients; Section 5.2.3. and Table 5.3). These 12 items were divided into those for which consumption is proposed to be beneficial in terms of AD risk and those for which the contrary is suggested. Participants were assigned a value of 1 for each of the proposed beneficial items if their consumption was at or above the sex-specific median intake level (total fish, olives, dark chocolate, total grapes, sesame seeds, sunflower seeds, total seeds, almonds and total nuts). By contrast, participants were assigned a value of 1 for each of the proposed detrimental items if their consumption was below the sex-specific median intake level (white rice). Total chocolate and total rice were not incorporated in the construction of the 'AD diet index', as elements of these food items are expected to be beneficial in terms of AD risk (dark chocolate and brown rice) whilst other elements are proposed to be detrimental with respect to AD risk (milk chocolate and white rice). The points were summed to produce a score ranging from 0 to 10, with a higher score proposed to be 'beneficial' in terms of AD risk. Table 2.9 lists the characteristics of the 'AD diet index'. Of the 49 individuals who participated in the validation study, and consequently for whom an 'AD diet index' was constructed, no one achieved an 'AD diet index' score of 0, 9 or 10. To further divide individuals into proposed 'higher' or 'lower' AD risk categories on the basis of 'AD diet index' scores, participants with a score of 1 – 4 were assigned a binary value of '1' to represent proposed 'higher' AD risk, and participants with a score of 5 – 8 were assigned a binary value of '0' to represent proposed 'lower' AD risk.

Table 2.9: *Characteristics of the 'Alzheimer's disease diet index'*

'Alzheimer's disease diet index'
High intake of total fish
High intake of olives
High intake of dark chocolate
High intake of total grapes
High intake of sesame seeds
High intake of sunflower seeds
High intake of total seeds
High intake of almonds
High intake of total nuts
Low intake of white rice

The mean neuropsychological test scores for a range of cognitive test MMSE, CVLT-II delayed recall, recognition and recognition discrimination, RCFT 3 minute delayed recall and COWAT; see section 2.10.4. for details of test administration and scoring protocols) were compared between the proposed 'low' and 'high' AD risk groups determined on the basis of 'AD diet index' adherence. Independent samples t-tests were initially used to compare means between the two AD risk groups for their neuropsychological test scores followed by general linear models in order to control for the most common confounding variables with respect to diet and AD research, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, and BMI. All analysis were cross-sectional, with the data from the assessment time-point closest to the date of the validation study utilised in the analysis.

Mean levels of the PathWest blood-based inflammatory biomarkers analysed in section 4.2.4.2. (haemoglobin, red blood cell count, erythrocyte sediment rate, platelet count, white cell count, neutrophils, lymphocytes, monocytes, eosinophils and basophils) were compared between the proposed 'high' and 'low' AD risk groups again determined on the basis of 'AD diet index' adherence using the same methods to those utilised for the cognition analysis undertaken in the cognition analysis in this section. (i.e. independent samples t-tests followed by general linear models controlling for common confounders).

Thirty seven of the 49 participants included in the validation study had undergone a PiB PET scan. Depending on their PiB PET SUVR they were classified as either PiB PET negative or PiB PET positive). In multinomial logistic regressions, this binary score (PiB PET positive 1, PiB PET negative 0) was used to calculate the odds of being PiB PET positive rather than PiB PET negative if individuals are in the proposed 'high' AD risk group compared to the 'low' AD risk group.

Chapter Three

The association of four dietary patterns with clinical classification and cognitive decline

3.1. Introduction

The world's population is growing older due to improved healthcare and nutrition. As a result, Alzheimer's disease (AD) prevalence is rapidly increasing. Cognitive decline is the progressive loss of cognitive functions, including memory, and may lead to dementia of which AD is the most common type, accounting for 60 - 80% of cases (279). The focus of the current research climate is shifting from understanding AD pathology and diagnosis to primary prevention and intervention strategies. Diet represents one potential intervention strategy accessible to all.

Individuals consume diets that contain both nutrient and non-nutrient substances rather than single foods. Consequently, misleading conclusions on the effect of consumption of a single nutrient, food or dietary component on health outcomes can be drawn. It may be more useful therefore to examine indices of food and nutrient intake that express several related aspects of diet concurrently rather than focus on consumption of single nutrients (280): as a result, dietary pattern analysis has emerged (219).

There are two methods to derive dietary patterns, '*a priori'* and '*a posteriori'*. '*A priori'* patterns are hypothesis-driven, they require prior knowledge of diet and diseases and make use of existing methods to calculate a dietary pattern (158): an example of this is the Mediterranean diet (MeDi). '*A posteriori'* patterns are exploratory in nature; they use statistical methods such as principle components analysis, cluster analysis or reduced rank regression to derive dietary patterns from previously collected data (243, 281).

Results from previous studies on the association between dietary patterns and cognitive decline are inconsistent. Several American and European studies have shown a positive relationship between adherence to the MeDi and slower cognitive decline or reduced AD risk (1-7). By contrast, studies have concluded that higher consumption of the MeDi is not protective against cognitive decline (226, 282, 283). Methodological and cohort variations may account for the contradictory results. Studies examining risk of cognitive decline using '*a posteriori'* developed dietary patterns are limited in number, they have generally found that higher consumption of food from an 'unhealthy' dietary pattern rich in processed foods is associated with increased cognitive decline with the converse true for a 'healthy' or 'whole food' dietary pattern (6, 7). To our knowledge, the association between food consumption from dietary patterns rich in either anti- or pro-

inflammatory foods with AD risk and cognitive decline has not previously been investigated. There is a need for further longitudinal investigation of potential associations between dietary patterns and cognitive decline as well as AD risk in well-characterised ageing cohorts: this study aims to address this need.

3.1.1. Methods

Our study utilised a well-characterised, elderly, Australian cohort, taken from the larger Australian Imaging, Biomarkers and Lifestyle study of ageing (AIBL; 11). All participants underwent an extensive neuropsychological assessment at baseline, 18 and 36 month follow-up. At each time point, individuals were classified by a clinical panel (comprising old age psychiatrists, a neurologist, a geriatrician and neuropsychologists) as cognitively healthy control, mild cognitive impairment (MCI) or AD. Using data collected via administration of the Cancer Council of Victoria food frequency questionnaire (CCVFFQ; see Section 2.9.1. for detailed methods) four dietary patterns were constructed. A MeDi and inflammatory dietary index were constructed using an '*a priori'* method and a western and prudent diet pattern by factor analysis (principle components); an '*a posteriori'* method. The MeDi is characterised by a high intake of vegetables, legumes, fruits, cereals, fish and unsaturated fatty acids, low intake of saturated fatty acids, meat and poultry, low-to-moderate intake of dairy products, and a regular but moderate intake of alcohol. The inflammatory dietary index includes intake of foods and nutrients thought to counteract inflammation, including niacin, thiamine, riboflavin, iron, magnesium, zinc, vitamin A, C and E, folate, beta-carotene, garlic and onion. A higher consumption of these components results in a lower inflammatory dietary index. The 'healthy' prudent diet pattern includes high consumption of vegetables, fruit and nuts. Whilst the 'unhealthy' western diet includes high consumption of red and processed meats, chips, refined grains, potatoes, sweets and condiments. The components of the four dietary patterns are summarised in Table 3.1.

Table 3.1: *Components of the four dietary patterns*

Mediterranean diet	**Inflammatory dietary index**	**Prudent diet**	**Western diet**
High intake of vegetables	Niacin	Vegetables	Red meat
High intake of legumes	Thiamine	Fruit	Processed meat
High intake of fruits	Riboflavin	Nuts	Chips
High intake of cereals	Iron	Whole grains	Refined grains
High intake of fish	Magnesium	Tomatoes	Poultry
High intake of unsaturated fatty acids	Zinc	Fish	Condiments
Low intake of saturated fatty acids	Vitamin A	Low fat dairy	Potatoes
Low intake of meat	Vitamin C	Potatoes	Sweets
Low intake of poultry	Vitamin E	Poultry	Breakfast cereals
Low to moderate intake of dairy products	Folate	Garlic	Meat pies
Regular but moderate intake of alcohol	Beta-carotene	Snacks	Margarine
	Garlic		High fat dairy
	Onion		Dark yellow vegetables
			Fruit juice
			Snacks
			Beer
			Hamburger
			Pizza

3.1.2. Chapter aims

This chapter utilises dietary data and neuropsychological assessment data collected as part of the longitudinal prospective AIBL study to address the following aims:

1) To investigate cross-sectionally the association of four dietary patterns; MeDi, inflammatory dietary index, western diet and prudent diet, with baseline clinical classification.

2) To investigate longitudinally the association of these four dietary patterns with cognition and cognitive change over a three year follow-up period, using a comprehensive battery of neuropsychological tests.

3) To assess longitudinally whether dietary pattern adherence influences the rate of transition from healthy control to MCI or AD clinical classification.

Whilst the literature is conflicting, we hypothesise that:

1) Healthy controls have a higher adherence to the MeDi and prudent diet than AD and MCI participants.

3) Healthy controls have a lower adherence to the western diet pattern and the inflammatory dietary index than AD and MCI participants.

4) Higher prudent diet and MeDi adherence is associated with less cognitive decline over 36 months on a range of cognitive assessments compared with lower prudent and MeDi adherence.

5) Higher western diet adherence and a higher inflammatory dietary index is associated with increased cognitive decline over 36 months on a range of cognitive assessments compared with lower western diet adherence and a lower inflammatory dietary index.

6) Higher prudent diet or MeDi adherence at baseline is associated with a decreased number of participants transitioning from healthy control clinical classification to MCI or AD after 36 months.

7) Higher western diet adherence and a higher inflammatory dietary index at baseline is associated with an increased number of participants transitioning from healthy control clinical classification to MCI or AD after 36 months.

3.2. Results

3.2.1 Descriptive statistics for the baseline clinical classification analysis

The adherence to dietary patterns and baseline clinical classification analysis included 723 healthy controls (HC), 98 MCI and 149 AD participants who completed the CCVFFQ at their baseline assessment. The cohort as a whole had an average age of 71.7 ± 7.9 years. Nearly half of the cohort was male (42%), and nearly half had 12 or less years of education (49%). The average body mass index (BMI) of the cohort at baseline was 26.2 ± 4.2 kilograms per metres squared (kg/m^2).

Inter-classification differences were apparent at baseline. Unsurprisingly, AD participants were older than both MCI and HC subjects ($p < 0.001$; ANOVA). The AD group also had a higher percentage of people carrying an Apolipoprotein (*APOE)* ε4 allele ($p < 0.001$; $\chi 2$), and a higher percentage of individuals with 12 or less years of education than the MCI and HC groups ($p = 0.007$; $\chi 2$). The HCs had a higher BMI than MCI and AD participants ($p < 0.001$; ANOVA). In regards to cardiovascular risk factors, the MCI group had a higher percentage of participants with a history of angina than the AD and HC groups ($p = 0.017$; $\chi 2$), and ADs had a higher percentage of people with a history of stroke than the HC and MCI groups ($p < 0.001$; $\chi 2$). The descriptive statistics of the cohort as a whole and following stratification of the cohort by clinical classification are shown in Table 3.2.

Table 3.2: *Descriptive statistics for the whole cohort and for HC, MCI and AD participants at baseline.*

	Whole cohort (n = 970)	HC (n = 723)	MCI (n = 98)	AD (n = 149)	*p*-values for inter-classification differences
Age; y	71.7 ± 7.9	69.9 ± 7.0	76.0 ± 7.8	77.5 ± 8.2	**0.000**
Gender; male; n (%)	407 (42)	303 (42)	41 (42)	63 (42)	0.996
Country of birth; Australian; n (%)	687 (71)	519 (72)	63 (64)	105 (70)	0.308
Presence of *APOE* ε4 allele; n (%)	331 (34)	186 (26)	52 (53)	93 (62)	**0.000**
Body mass index[i]; kg/m^2	26.2 ± 4.2	26.5 ± 4.2	25.6 ± 3.8	24.8 ± 4.3	**0.000**
Energy intake; kCal	1695 ± 599	1691 ± 594	1710 ± 618	1702 ± 616	0.942
Diabetes; n (%)[ii]	76 (8)	51 (7)	11 (11)	14 (8)	0.263
Hypertension; n (%)[ii]	387 (40)	292 (40)	39 (40)	56 (38)	0.817
Angina; n (%)[ii]	70 (7)	43 (6)	13 (13)	14 (9)	**0.017**
Heart attack; n (%)[ii]	50 (5)	33 (5)	7 (7)	10 (7)	0.360
Stroke; n (%)[ii]	33 (3)	14 (2)	2 (2)	17 (11)	**0.000**
Current smoker; n (%)	29 (3)	25 (3)	4 (4)	0 (0)	0.063
Education ≤ 12 y; n (%)	447 (49)	355 (46)	53 (54)	89 (60)	**0.007**

Unless otherwise stated, data are presented as mean ± standard deviation of the mean. Bold indicates statistical significance ($p < 0.05$); Characteristics compared using analysis of variance for continuous variables and χ^2 for categorical variables. [i]Body mass index is calculated as weight in kilograms divided by height in meters squared. [ii]History of diabetes, hypertension, angina, heart attack or stroke determined by participant self-reported medical history. Abbreviations: *APOE*, Apolipoprotein E; AD, Alzheimer's disease; HC, healthy control; kCal, kilocalorie; Kg, kilogram; m^2, meter squared; MCI, mild cognitive impairment; y, years.

3.2.2 Comparison of diet scores and clinical classification groups

Mean scores were calculated for each of the four dietary patterns both for the cohort as a whole at baseline and following stratification of the cohort by clinical classification. For the cohort as a whole, the mean MeDi score at baseline was 4.4 ± 1.6, mean prudent score was 234.8 ± 121.7, mean western diet score was 133.2 ± 122.3, and mean inflammatory dietary index was 0.83 ± 1.5. Following stratification by clinical classification, a significant difference in all four mean dietary pattern scores was observed between the three clinical classification groups ($p < 0.01$; ANOVA; Table 3.3).

To further explore the relationship between adherence to each of the four dietary patterns and clinical classification at baseline, we used multinomial logistic regressions. The most common confounding variables were controlled for in all analyses, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, energy intake, smoking status, and history of hypertension, angina, stroke, diabetes and heart attack: these variables can affect the outcome of the analysis and failure to include them in the models can cause misestimates of relationships.

We found that healthy control participants had a higher adherence to the MeDi than participants with AD or MCI ($p < 0.001$ and $p < 0.05$, respectively; Figure 3.1A). There was no difference in MeDi adherence between MCI and AD participants. Healthy control participants also had a higher adherence to the prudent diet than participants with AD or MCI ($p < 0.001$ and $p < 0.05$, respectively), and in this case, a difference was observed between MCI and AD participants with the MCI group demonstrating higher adherence to the prudent diet than participants with AD ($p < 0.05$; Figure 3.1B). In contrast, healthy control and MCI participants had a lower western diet score than AD participants ($p < 0.001$ and $p < 0.01$, respectively; Figure 3.1C) with no difference in western diet adherence observed between healthy control and MCI participants. Further, healthy control participants had a higher consumption of anti-inflammatory foods (indicated by a lower mean inflammatory dietary index) than those with AD ($p < 0.001$) and MCI ($p < 0.01$), whilst there was no difference in the inflammatory index between AD and MCI participants (Figure 3.1D).

Table 3.3: *Mean dietary pattern scores for the whole cohort and for HC, MCI and AD participants at baseline.*

	Whole cohort (n = 970)	HC (n = 723)	MCI (n = 98)	AD (n = 149)	*p*-values for inter-classification differences
MeDi score	4.4 ± 1.6	4.6 ± 1.6	4.0 ± 1.6	3.8 ± 1.6	**0.000**
Prudent diet score	234.8 ± 121.7	250.0 ± 118.0	211.8 ± 127.6	176.5 ± 116.1	**0.000**
Western diet score	133.2 ± 122.3	126.8 ± 124.4	132.0 ± 116.8	165.0 ± 110.4	**0.002**
Inflammatory dietary index	0.83 ± 1.5	0.72 ± 1.4	1.05 ± 1.5	1.23 ± 1.6	**0.000**

Data are presented as mean ± standard deviation of the mean. Bold indicates statistical significance ($p < 0.01$); Characteristics compared using analysis of variance for continuous variables. Abbreviations: AD, Alzheimer's disease; HC, healthy control; MCI, mild cognitive impairment; MeDi, Mediterranean diet.

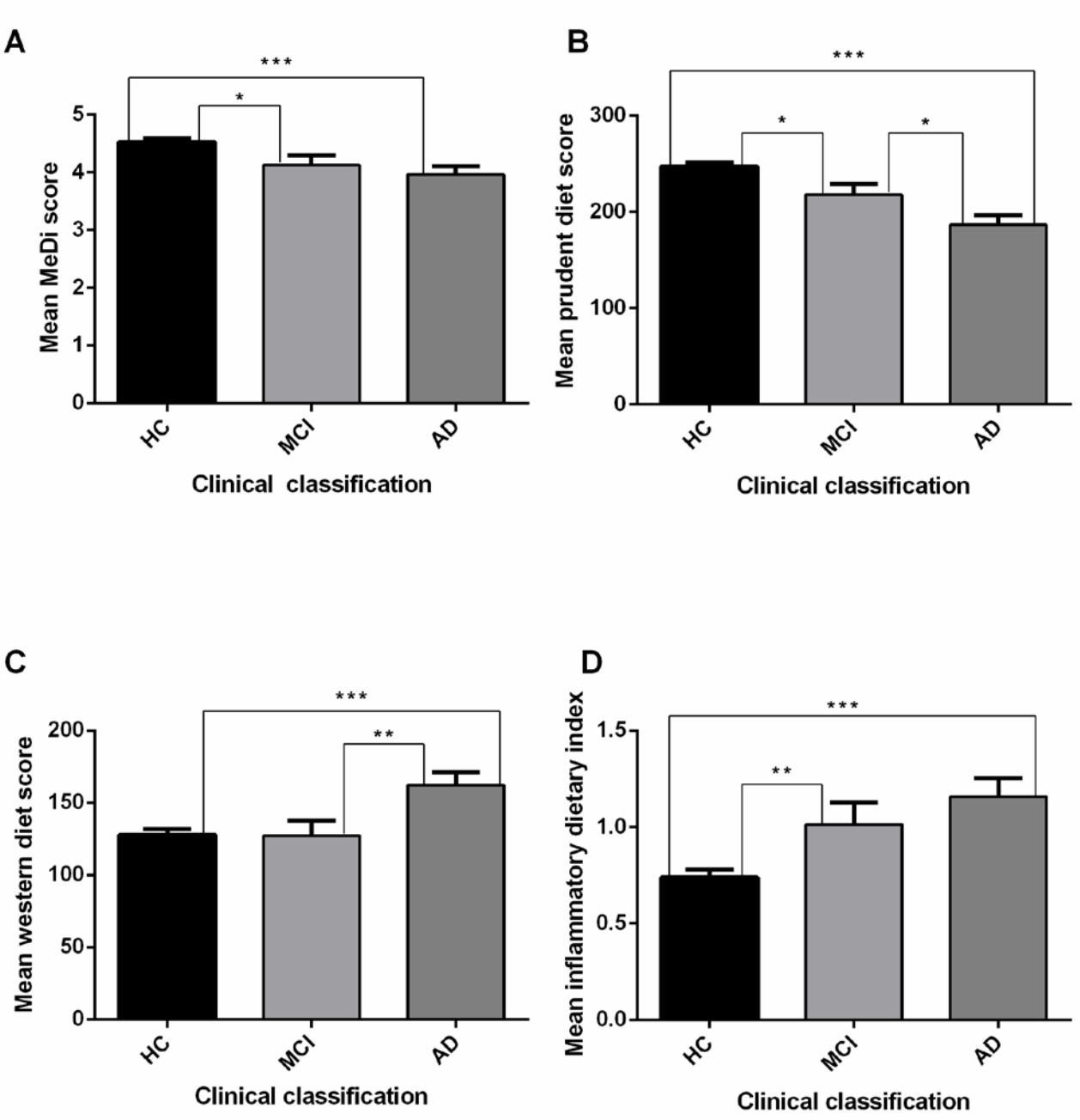

Figure 3.1: *Comparison of mean diet scores for HC, MCI and AD participants at baseline using multinomial logistic regressions adjusted for necessary covariates. A – MeDi score, B – prudent diet score, C – western diet score, D – inflammatory dietary index.*

Bars represent mean (± standard error). Models adjusted for age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), body mass index, energy intake kCal, current smoking status, and history of hypertension, angina, stroke, diabetes and heart attack. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: *APOE*, Apolipoprotein E; AD, Alzheimer's disease; HC, healthy control; kCal, kilocalorie; MCI, mild cognitive impairment; MeDi, Mediterranean diet.

These results suggest that AD participants in our study had a higher adherence to the 'unhealthy' western diet pattern, lower consumption of anti-inflammatory foods, and a lower adherence to the 'healthy' MeDi and prudent dietary patterns at baseline compared to cognitively 'healthy' controls. However, this analysis is cross-sectional

and therefore we cannot conclude that decreased adherence to a 'healthy' dietary pattern or conversely increased adherence to an 'unhealthy' dietary patterns is a risk factor for AD. It is possible that AD diagnosis may result in a change of dietary habits. Furthermore, we are using an FFQ that requires estimations of food intake over the previous year, there is potential therefore for misclassification due to limited accuracy in estimations, particularly with regard to MCI and AD participants. Consequently, from this point onwards, to assess the relationship between diet and cognitive decline, we will only utilise data collected from individuals classified as healthy control at baseline.

3.2.3 Descriptive statistics for the healthy control cohort

All healthy control participants with a completed CCVFFQ at baseline and cognitive testing at three time points (baseline, 18 and 36 month follow-up) were included in the following analyses assessing cognition and cognitive decline: this decreased the number of healthy control participants included in subsequent analysis from 723 (sections 3.2.1. and 3.2.2.) to 527.

The average age of the healthy control cohort at baseline was 69.3 ± 6.4 and nearly 40% were male. Twenty eight per cent had at least one *APOE* ε4 allele, over 45% had 12 or less years of education and nearly 80% were born in Australia. With respect to the cardiovascular risk factors; 7.2% had diabetes, 38.1% had a history of hypertension, 5.7% had a history of angina, 4.9% had a history of heart attack, 1.9% had previously suffered a stroke and 43.3% were past smokers. Mean BMI was 26.3 ± 4.2 and mean energy intake was 1708 kCal. The average baseline MeDi score was 4.6 ± 1.6, the average prudent diet score was 307.5 ± 130.0, the average western diet score was 144.3 ± 148.9, and the average inflammatory dietary index was 0.67 ± 1.4 (Table 3.4).

Due to reported associations between cognition and gender, the healthy control cohort was also assessed following stratification by gender. There were several differences observed between males and females. Females had a higher percentage of people born in Australia ($p = 0.014$; χ^2), a higher percentage with 12 or less years of education ($p = 0.020$; χ^2) and a lower mean BMI ($p = 0.032$; ANOVA). As expected, males had a higher energy intake ($p < 0.001$; ANOVA). Amongst the male group there was also a higher percentage of people with a history of angina, a history of heart attack and who

are past smokers ($p = 0.020$, $p = 0.021$ and $p < 0.001$, respectively; χ^2). With respect to the diet scores, the western diet score was significantly higher in males and the inflammatory dietary index was significantly higher in females ($p < 0.001$; ANOVA; Table 3.4).

When the healthy control participants were stratified by *APOE* ε4 allele carriage (the major genetic risk factor for AD), the only difference between *APOE* ε4 allele carriers and non-carriers was the prudent diet score which was higher in non-carriers ($p = 0.017$; ANOVA; Table 3.4).

Table 3.4: *Descriptive statistics for the healthy control cohort as a whole, and subgroups based on stratification of the cohort by gender and APOE ε4 allele carriage.*

Characteristic	Whole cohort (527)	Males (210)	Females (317)	*p*-values for gender differences	*APOE* ε4 carrier (148)	*APOE* ε4 non-carrier (379)	*p*-values for *APOE* ε4 carriage differences
Age at baseline; y	69.3 ± 6.4	69.7 ± 6.2	69.0 ± 6.6	0.250	68.4 ± 6.1	69.6 ± 6.5	0.054
Gender; men; n (%)	210 (39.8)				61 (41.2)	149 (39.3)	0.689
Country of birth; Australian; n (%)	417 (79.1)	155 (73.8)	262 (82.6)	**0.014**	120 (81.1)	297 (78.4)	0.490
Presence of *APOE* ε4 allele; n (%)	148 (28.1)	61 (29.0)	87 (27.4)	0.689			
Baseline body mass index[i]; kg/m²	26.3 ± 4.2	26.7 ± 3.8	26.0 ± 4.4	**0.032**	26.0 ± 4.2	26.4 ± 4.1	0.340
Baseline energy intake; kCal	1708.1 ± 557.2	1972.5 ± 591.7	1532.9 ± 456.0	**0.000**	1726.1 ± 577.7	1701.1 ± 549.6	0.644
Diabetes; n (%)[ii]	38 (7.2)	16 (7.6)	22 (6.9)	0.768	13 (8.8)	25 (6.6)	0.383
Hypertension; n (%)[ii]	201 (38.1)	75 (35.7)	126 (39.7)	0.351	51 (34.5)	150 (39.6)	0.277
Angina; n (%)[ii]	30 (5.7)	18 (8.6)	12 (3.8)	**0.020**	10 (6.8)	20 (5.3)	0.510
Heart Attack; n (%)[ii]	26 (4.9)	16 (7.6)	10 (3.2)	**0.021**	9 (6.1)	17 (4.5)	0.447
Stroke; n (%)[ii]	10 (1.9)	6 (2.9)	4 (1.3)	0.189	3 (2.0)	7 (1.8)	0.892
Past smoker; n (%)	228 (43.3)	112 (53.3)	116 (36.6)	**0.000**	66 (44.6)	162 (42.7)	0.700
Education ≤ 12 y; n (%)	241 (45.7)	83 (39.5)	158 (49.8)	**0.020**	77 (52.0)	164 (43.3)	0.070
Baseline MeDi score	4.6 ± 1.6	4.6 ± 1.7	4.6 ± 1.6	0.881	4.7 ± 1.5	4.5 ± 1.7	0.164
Baseline prudent diet score	307.5 ± 130.0	306.9 ± 146.1	307.9 ± 118.4	0.931	285.9 ± 116.1	316.0 ± 134.3	**0.017**
Baseline western diet score	144.3 ± 148.9	218.5 ± 175.2	95.2 ± 102.7	**0.000**	160.8 ± 152.2	137.9 ± 147.3	0.113
Baseline inflammatory dietary index	0.67 ± 1.4	0.33 ± 1.4	0.90 ± 1.4	**0.000**	0.83 ± 1.4	0.61 ± 1.4	0.119

Unless otherwise stated, data are presented as mean ± standard deviation of the mean. Bold indicates statistical significance ($p < 0.05$); Characteristics compared using analysis of variance for continuous variables and χ^2 for categorical variables. [i]Body mass index is calculated as weight in kilograms divided by height in meters squared. [ii]History of diabetes, hypertension, angina, heart attack or stroke determined by participant self-reported medical history. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; Kg, kilogram; m^2, meter squared; MeDi, Mediterranean diet; y, years.

3.2.4 Correlation of the four diet scores at baseline

Having constructed MeDi, inflammatory dietary index, prudent and western diet scores for each healthy control participant at baseline, bivariate Pearson's correlations were then used to evaluate how the four dietary patterns related to one another. A positive correlation indicates that as one score increases, so does the other, whilst a negative correlation indicates that as one score increases the other decreases. The previous analysis was exploratory in nature and for this reason we used a *p*-value of 0.05 or smaller to determine a significant result. For all subsequent analysis a *p*-value of 0.01 or smaller determined a significant result to balance the risk of type I and type II errors, due to the large number of statistical tests performed.

As expected, there was a positive correlation between prudent diet score and MeDi score ($p < 0.001$): both are 'healthy' eating patterns. Prudent diet score was also negatively correlated with western diet score ($p < 0.001$); suggesting that as a person eats more foods in the prudent ('healthy') dietary pattern, they eat less foods in the western ('unhealthy') dietary pattern. Unexpectedly, MeDi score was not correlated with western diet score. The inflammatory dietary index was negatively correlated with MeDi and prudent dietary patterns ($p < 0.001$), suggesting that as consumption of anti-inflammatory foods decreases (increasing inflammatory dietary index), adherence to 'healthy' dietary patterns also decreases, however somewhat surprisingly, the inflammatory dietary index was also negatively correlated with western diet score ($p < 0.001$; Table 3.5).

After stratifying by gender and *APOE* ε4 allele carriage, these correlations remained consistent (data not shown).

Table 3.5: *Correlation matrix of the four diet scores.*

	MeDi score	Prudent score	Western score	Inflammatory dietary index
MeDi score		**0.265**	0.046	**-0.287**
Prudent score	**0.265**		**-0.339**	**-0.644**
Western score	0.046	**-0.339**		**-0.181**
Inflammatory dietary index	**-0.287**	**-0.644**	**-0.181**	

Data is presented as r values from bivariate Pearson's correlations. Bold indicates statistical significance. Abbreviations: MeDi, Mediterranean diet.

3.2.5. Cognitive performance of the healthy control cohort

Prior to assessing the relationship between dietary patterns and cognition, we assessed how cognitive performance changed over the 36 month period of follow-up, and determined if there were differences in performance between the genders, and *APOE* ε4 allele carriers and non-carriers. From the comprehensive neuropsychological test battery administered at baseline, 18 and 36 month follow-up, composite scores were constructed for six cognitive domains and a global cognitive score was also computed using these six domains. The six domains assessed were; verbal and visual memory, attention, language, executive function and visuospatial functioning (detailed methods of constructing these composites can be found in Section 2.10.12).

All mean cognitive composite domain scores (and therefore the global cognitive score) were higher at baseline than at 36 month follow-up; a decline in cognitive performance over 36 months is expected amongst the demographic assessed. There were several gender-related differences between performances in the cognitive domains assessed. Females performed better in the verbal memory domain at baseline ($p < 0.001$; ANOVA), and in the attention domain at 36 month follow-up ($p = 0.005$; ANOVA). Whilst males performed better in the visual memory domain at both time points ($p = 0.002$ at baseline and $p = 0.004$ at 36 month follow-up; ANOVA). There were no differences between males and females in their global cognitive score at baseline or 36 month follow-up. There were no differences between *APOE* ε4 allele carriers and non-carriers in any of the cognitive domains assessed at baseline or 36 month follow-up. The

cognitive results over 36 months of the healthy cohort as a whole and following stratification by gender and *APOE* ε4 allele carriage are shown in Table 3.6.

Table 3.6: *Cognitive performance for the whole healthy control cohort at baseline and 36 month follow-up, and for subgroups following stratification of the cohort by gender and APOE ε4 allele carriage.*

Cognitive Domain	Time point[i]	Total Sample	n	Males	n	Females	n	*p*-values for gender differences	*APOE* ε4 allele carrier	N	*APOE* ε4 allele non-carrier	n	*p*-values for *APOE* ε4 carriage differences
Verbal memory	1	0.06 ± 0.78	527	-0.15 ± 0.78	210	0.20 ± 0.75	317	**0.000**	0.05 ± 0.78	148	0.06 ± 0.79	379	0.854
Visual memory	1	0.03 ± 0.81	527	0.17 ± 0.84	210	-0.06 ± 0.79	317	**0.002**	-0.03 ± 0.82	148	0.06 ± 0.81	379	0.298
Attention	1	0.04 ± 0.69	527	-0.01 ± 0.67	210	0.06 ± 0.70	317	0.246	-0.04 ± 0.74	148	0.07 ± 0.67	379	0.115
Language	1	0.06 ± 0.88	527	0.06 ± 0.70	210	0.06 ± 0.86	317	0.915	-0.03 ± 0.86	148	0.10 ± 0.77	379	0.110
Executive function	1	0.02 ± 0.60	527	-0.05 ± 0.56	210	0.07 ± 0.61	317	0.024	-0.04 ± 0.63	148	0.04 ± 0.58	379	0.197
Visuospatial functioning	1	0.05 ± 0.74	527	0.04 ± 0.82	210	0.06 ± 0.69	317	0.820	0.05 ± 0.74	148	0.05 ± 0.74	379	0.971
Global cognitive score	1	0.04 ± 0.46	527	0.01 ± 0.45	210	0.07 ± 0.47	317	0.152	-0.01 ± 0.50	148	0.06 ± 0.45	379	0.162
Verbal memory	3	-0.23 ± 0.99	525	-0.32 ± 1.07	210	-0.16 ± 0.94	315	0.069	-0.36 ± 1.15	148	-0.17 ± 0.92	377	0.051
Visual memory	3	-0.16 ± 0.87	523	0.29 ± 0.85	210	0.07 ± 0.88	313	**0.004**	0.08 ± 0.97	147	0.19 ± 0.83	376	0.205
Attention	3	-0.07 ± 0.72	525	-0.18 ± 0.80	210	0.00 ± 0.66	315	**0.005**	-0.15 ± 0.72	148	-0.04 ± 0.72	377	0.125
Language	3	-0.02 ± 0.88	523	-0.00 ± 0.77	210	-0.04 ± 0.95	313	0.648	-0.18 ± 1.07	147	0.04 ± 0.79	376	0.013
Executive function	3	-0.16 ± 0.60	524	-0.24 ± 0.58	210	-0.11 ± 0.61	314	0.019	-0.22 ± 0.62	148	-0.14 ± 0.59	376	0.210
Visuospatial functioning	3	-0.39 ± 0.92	525	-0.35 ± 0.95	210	-0.41 ± 0.90	315	0.414	-0.40 ± 1.04	148	-0.38 ± 0.87	377	0.883
Global cognitive score	3	-0.11 ± 0.56	322	-0.13 ± 0.57	210	-0.10 ± 0.55	312	0.445	-0.18 ± 0.62	146	-0.08 ± 0.53	376	0.105

Unless otherwise stated, data are presented as mean ± standard deviation of the mean, with a higher score indicating better performance. Bold indicates statistical significance ($p < 0.01$). Characteristics compared using analysis of variance for continuous variables. [i]Time point 1 is baseline, time point 3 is 36 month follow-up. Abbreviations: *APOE*, Apolipoprotein E.

3.2.6. Diet score and cognitive function

Multiple linear regression analyses were run to investigate the cross-sectional association between baseline diet scores and baseline cognitive performance. One potential mechanism through which diet has been proposed to exert its effect is via the vascular system (104, 284, 285). To investigate the influence of vascular variables in our population, consistent with the analysis in section 3.2.2., we analysed an initial model with the most common confounding variables controlled for, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, energy intake, smoking status, and history of hypertension, angina, stroke, diabetes and heart attack, and additionally analysed a second model with the same confounding variables as the initial model without the inclusion of the vascular risk factors, i.e. smoking status, and history of hypertension, angina, stroke, diabetes and heart attack. We saw no associations in the linear regressions between diet scores and baseline composite scores in the six cognitive domains assessed or the global cognitive score, in the cohort as a whole (Table 3.7) or following stratified of the cohort by sex or *APOE* ε4 allele carriage (Table 3.8 and 3.9).

Table 3.7: *Relationship between baseline diet scores and baseline cognitive test scores: linear regression analysis. Standardised β values shown.*

Model	1	2	1	2	1	2	1	2
Composite cognitive domain	**Baseline MeDi score**		**Baseline prudent diet score**		**Baseline western diet score**		**Baseline inflammatory dietary index**	
Verbal memory	0.015	0.014	-0.000	-0.000	0.000	0.000	0.047	0.048
Visual memory	-0.024	-0.025	-0.000	-0.000	0.000	0.000	-0.043	0.010
Executive function	0.023	0.023	0.000	0.000	0.000	0.000	0.010	0.010
Language	0.009	0.009	0.001	0.001	-0.000	-0.000	-0.040	-0.041
Attention	0.019	0.019	0.000	0.000	-0.000	-0.000	0.077	0.072
Visuospatial functioning	-0.018	-0.018	0.000	0.000	-0.000	-0.000	-0.073	-0.074
Global cognitive score	0.004	0.000	0.000	0.000	-0.000	-0.000	-0.006	0.005

Model 1 - adjusted model includes age, gender, *APOE* ε4 allele carrier status, years of education, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 3.8: *Relationship between baseline diet scores and baseline cognitive test scores: linear regression analysis following stratification of the cohort by gender. Standardised β values shown.*

Model		1	2	1	2	1	2	1	2
Composite cognitive domain	**Gender[a]**	**Baseline MeDi score**		**Baseline prudent diet score**		**Baseline western diet score**		**Baseline inflammatory dietary index**	
Verbal memory	M	0.011	0.009	-0.000	-0.000	0.000	0.000	0.076	0.078
Visual memory	M	0.007	-0.001	-0.000	0.000	-0.000	-0.000	-0.076	-0.060
Executive function	M	0.035	0.036	-0.000	-0.000	0.000	0.000	0.077	0.078
Language	M	0.008	0.005	0.000	0.000	0.000	0.000	-0.072	-0.073
Attention	M	0.033	0.029	-0.000	-0.000	-0.000	-0.000	0.113	0.167
Visuospatial functioning	M	-0.035	-0.040	0.001	0.001	-0.001	-0.000	-0.074	-0.075
Global cognitive score	M	0.010	0.006	-0.000	0.000	-0.000	-0.000	0.008	0.020
Verbal memory	F	0.029	0.029	0.000	-0.000	-0.000	-0.000	0.026	0.026
Visual memory	F	-0.045	-0.041	-0.001	-0.001	0.001	0.001	0.015	0.083
Executive function	F	0.008	0.011	0.000	0.000	-0.000	-0.000	-0.009	-0.009
Language	F	0.015	0.017	0.001	0.001	-0.001	-0.001	-0.021	-0.021
Attention	F	0.007	0.011	0.000	0.000	-0.000	-0.000	0.119	0.035
Visuospatial functioning	F	0.003	0.010	0.000	0.000	0.000	0.000	-0.063	-0.063
Global cognitive score	F	0.029	0.029	0.000	-0.000	-0.000	-0.000	0.026	0.026

[a]Gender - M is male, F is female. Model 1 - adjusted model includes age, *APOE* ε4 allele carrier status, years of education, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 3.9: *Relationship between baseline diet scores and baseline cognitive test scores: linear regression analysis following stratification of the cohort by APOE ε4 allele carriage. Standardised β values shown.*

Model		1	2	1	2	1	2	1	2
Composite cognitive domain	***APOE* ε4 allele carriage[a]**	**Baseline MeDi score**		**Baseline prudent diet score**		**Baseline western diet score**		**Baseline inflammatory dietary index**	
Verbal memory	-	0.006	0.007	-0.000	-0.000	0.000	0.000	0.015	0.017
Visual memory	-	-0.039	-0.037	-0.000	-0.000	-0.000	-0.000	-0.031	0.031
Executive function	-	0.005	0.007	0.000	0.000	-0.000	-0.000	0.031	0.032
Language	-	0.001	0.004	0.000	0.001	-0.000	-0.000	-0.024	-0.025
Attention	-	0.005	0.006	0.000	0.000	-0.000	-0.000	0.112	0.077
Visuospatial functioning	-	-0.017	-0.014	0.000	0.000	-0.000	-0.000	-0.068	-0.069
Global cognitive score	-	0.038	0.033	0.000	0.000	0.000	0.000	0.007	0.014
Verbal memory	+	0.056	0.055	0.001	0.000	-0.001	-0.000	0.192	0.192
Visual memory	+	0.016	0.009	-0.001	-0.001	0.001	0.001	-0.025	0.005
Executive function	+	0.066	0.070	-0.000	-0.000	0.000	0.000	0.031	0.031
Language	+	0.030	0.030	0.001	0.001	-0.000	-0.000	-0.082	-0.082
Attention	+	0.067	0.052	-0.000	-0.000	0.000	0.000	0.036	0.107
Visuospatial functioning	+	-0.009	-0.019	0.000	0.000	0.000	0.000	-0.054	-0.054
Global cognitive score	+	0.038	0.033	0.000	0.000	0.000	0.000	0.025	0.047

[a]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model 1 - adjusted model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Having completed cross-sectional assessment of the relationship between the four diet scores and cognition, we sought to determine whether there was an association between diet score at baseline and cognitive performance over 36 months. Linear mixed models were used to analyse the relationship between MeDi, inflammatory dietary index, prudent and western diet, the six cognitive domains assessed and the global cognitive score in the cohort as a whole and following stratification by gender and *APOE* ε4 allele carriage. Linear mixed models use the baseline, 18 and 36 month follow-up cognitive domain scores in their analysis. The same two models utilised for the cross-sectional analysis were used for the longitudinal analysis; the initial model had the most common confounding variables controlled for, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, energy intake, smoking status, and history of hypertension, angina, stroke, diabetes and heart attack, and the second model had the same confounding variables as the initial model without the inclusion of the vascular risk factors, i.e. smoking status, and history of hypertension, angina, stroke, diabetes and heart attack. In both models there was a trend towards increased MeDi adherence and decreased decline in executive function composite score in the cohort as a whole ($p = 0.040$; Table 3.10). Subsequent analyses following stratification by *APOE* ε4 allele genotype indicated this relationship was only significant amongst *APOE* ε4 allele carriers ($p < 0.01$; Table 3.12 and Figure 3.2); i.e. a higher MeDi score was associated with less decline in executive function over three years in *APOE* ε4 allele carriers. Diet explained 8.1% of the variance, increasing to 8.6% without CVD risk factors. Even though 8.6% is a relatively small portion of the fixed effect variance in executive function composite score, the other statistically significant variables (age, years of education and history of angina) contributed less, explaining only 5.6%, 5.6% and 6.4% respectively. This means that of the observed MeDi score association with executive function amongst the *APOE* ε4 allele carriers, 8.1% of the relationship was explained by diet adherence, 5.6% was explained by age, 5.6% by education of the individual, 6.4% by a history of angina, and the remaining 74.3% by other factors.

In both models there was a negative trend towards western diet adherence and change in the visuospatial functioning composite score ($p = 0.022$; Table 3.10), an association which we subsequently determined was driven by *APOE* ε4 allele non-carriers ($p < 0.01$; Table 3.12 and Figure 3.3); i.e. a higher western diet score was associated with

more decline in visuospatial functioning over three years in *APOE* ε4 allele non-carriers. Western diet explained 3.6% of the variance, and decreased to 3.4% without CVD risk factors. By comparison, the other statistically significant variables (age, baseline BMI and years of education) explained 14.9%, 1.8% and 9.6% respectively of the fixed effect variance in visuospatial functioning composite score.

No significant relationships between prudent diet adherence, inflammatory dietary index and cognition were found, however, there was a negative trend towards inflammatory dietary index and change in the visuospatial functioning composite score in *APOE* ε4 allele carriers (p = 0.021); i.e. lower consumption of anti-inflammatory foods (higher inflammatory dietary index) was trending towards more decline in visuospatial functioning over three years in *APOE* ε4 allele carriers. There were no significant associations between diet scores and cognition following stratification by gender (Table 3.11). Furthermore, the global cognitive score was not associated with adherence to any of the four dietary patterns in the cohort as a whole or following stratification by gender and *APOE* ε4 allele carriage (Table 3.10 – 3.12).

Table 3.10: *Results of linear mixed model analyses examining the association between baseline diet scores and change in cognitive performance over 36 months. Standardised β values shown.*

Model	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Change in composite cognitive domain	**Baseline MeDi score**		**Baseline prudent diet score**		**Baseline western diet score**		**Baseline inflammatory dietary index**	
Verbal memory	0.0157	0.0154	0.0001	0.0001	-0.0002	-0.0002	0.0086	0.0055
Visual memory	-0.0085	-0.0091	-0.0000	-0.0002	-0.0002	-0.0001	-0.0174	-0.0206
Executive function	0.0261	0.0265	0.0000	0.0002	-0.0000	0.0000	0.0041	0.0010
Language	0.0013	0.0015	0.0002	0.0002	-0.0002	-0.0002	-0.0060	-0.0082
Attention	0.0089	0.0097	-0.0000	0.0007	-0.0002	-0.0002	0.0337	0.0291
Visuospatial functioning	-0.0267	-0.0269	0.0003	0.0004	-0.0005	-0.0004	-0.0494	-0.0527
Global cognitive score	0.0010	0.0009	0.0001	0.0002	-0.0002	-0.0002	-0.0055	-0.0084

Model 1 - adjusted model includes age, gender, *APOE* ε4 allele carrier status, years of education, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 3.11: *Results of linear mixed model analyses examining the association between baseline diet scores and change in cognitive performance over 36 months following stratification of the cohort by gender. Standardised β values shown.*

Model	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Gender	**Male**	**Male**	**Female**	**Female**	**Male**	**Male**	**Female**	**Female**	**Male**	**Male**	**Female**	**Female**	**Male**	**Male**	**Female**	**Female**
Change in composite cognitive domain	**Baseline MeDi score**				**Baseline prudent diet score**				**Baseline western diet score**				**Baseline inflammatory dietary index**			
Verbal memory	0.0262	0.0242	0.0153	0.0133	0.0003	0.0003	0.0000	-0.0000	-0.0001	-0.0001	-0.0004	-0.0003	-0.0006	-0.0090	0.0154	0.0156
Visual memory	0.0084	0.0041	-0.0173	-0.0148	0.0001	0.0002	-0.0002	-0.0002	-0.0003	-0.0003	0.0000	0.0000	-0.0483	-0.0523	0.0055	0.0028
Executive function	0.0414	0.0424	0.0138	0.0167	0.0001	0.0001	-0.0001	-0.0000	-0.0000	0.0000	0.0000	-0.0000	0.0097	0.0047	0.0009	-0.0054
Language	0.0041	0.0035	0.0067	0.0054	0.0001	0.0001	0.0002	0.0003	-0.0002	-0.0001	-0.0001	-0.0003	-0.0104	-0.0150	0.0026	-0.0087
Attention	0.0247	0.0228	-0.0013	-0.0001	-0.0003	-0.0002	0.0001	0.0002	-0.0002	-0.0002	-0.0001	-0.0002	0.0534	0.0463	0.0316	0.0209
Visuospatial functioning	-0.0421	-0.0517	-0.0061	-0.0025	0.0005	0.0005	0.0003	0.0003	-0.0005	-0.0004	-0.0006	-0.0006	-0.0679	-0.0790	-0.0285	-0.0314
Global cognitive score	0.0104	0.0075	0.0013	0.0030	0.0001	0.0002	0.0000	0.0001	-0.0002	-0.0002	-0.0002	-0.0002	-0.0107	-0.0174	0.0023	-0.0024

Model 1 - adjusted model includes age, years of education, *APOE* ε4 allele carrier status, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 3.12: *Results of linear mixed model analyses examining the association between baseline diet scores and change in cognitive performance over 36 months following stratification of the cohort by APOE ε4 allele carrier status. Standardised β values shown.*

Model	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
***APOE* ε4 allele carriage**[a]	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Change in composite cognitive domain	**Baseline MeDi score**				**Baseline prudent diet score**				**Baseline western diet score**				**Baseline inflammatory dietary index**			
Verbal memory	0.0149	0.0170	0.0368	0.0297	0.0000	0.0001	0.0003	0.0003	-0.0001	-0.0001	-0.0003	-0.0003	0.0936	0.0919	-0.0091	-0.0157
Visual memory	-0.0164	-0.0142	0.0213	0.0119	-0.0000	-0.0000	-0.0001	-0.0001	-0.0003	-0.0003	0.0005	0.0005	-0.0215	-0.0213	-0.0112	-0.0166
Executive function	0.0077	0.0101	**0.0772* (8.1%[ł])**	**0.0815** (8.6%[ł])**	0.0000	0.0001	-0.0001	-0.0001	-0.0001	-0.0001	0.0003	0.0003	0.0207	0.0144	0.0066	-0.0008
Language	-0.0069	-0.0041	0.0272	0.0276	0.0001	0.0001	0.0004	0.0003	-0.0002	-0.0002	0.0000	-0.0000	-0.0038	-0.0040	-0.0041	-0.0119
Attention	0.0014	0.0026	0.0427	0.0289	-0.0000	-0.0000	-0.0000	0.0000	-0.0003	-0.0003	0.0004	0.0003	0.0715	0.0631	0.0292	0.0253
Visuospatial functioning	-0.0110	-0.0090	-0.0584	-0.0690	0.0004	0.0004	0.0001	0.0001	**-0.0006* (3.6%[ł])**	**-0.0006* (3.4%[ł])**	0.0001	0.0001	-0.0100	-0.0085	-0.0570	-0.0611
Global cognitive score	-0.0023	-0.0002	0.0252	0.0200	0.0001	0.0001	0.0001	0.0001	-0.0003	-0.0003	0.0002	0.0002	0.0195	0.0184	-0.0073	-0.0132

[a]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model 1 - adjusted model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2 - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. [ł]Portion of variance explained by diet score. Bold indicates statistical significance (* $p < 0.01$, ** $p < 0.001$). Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

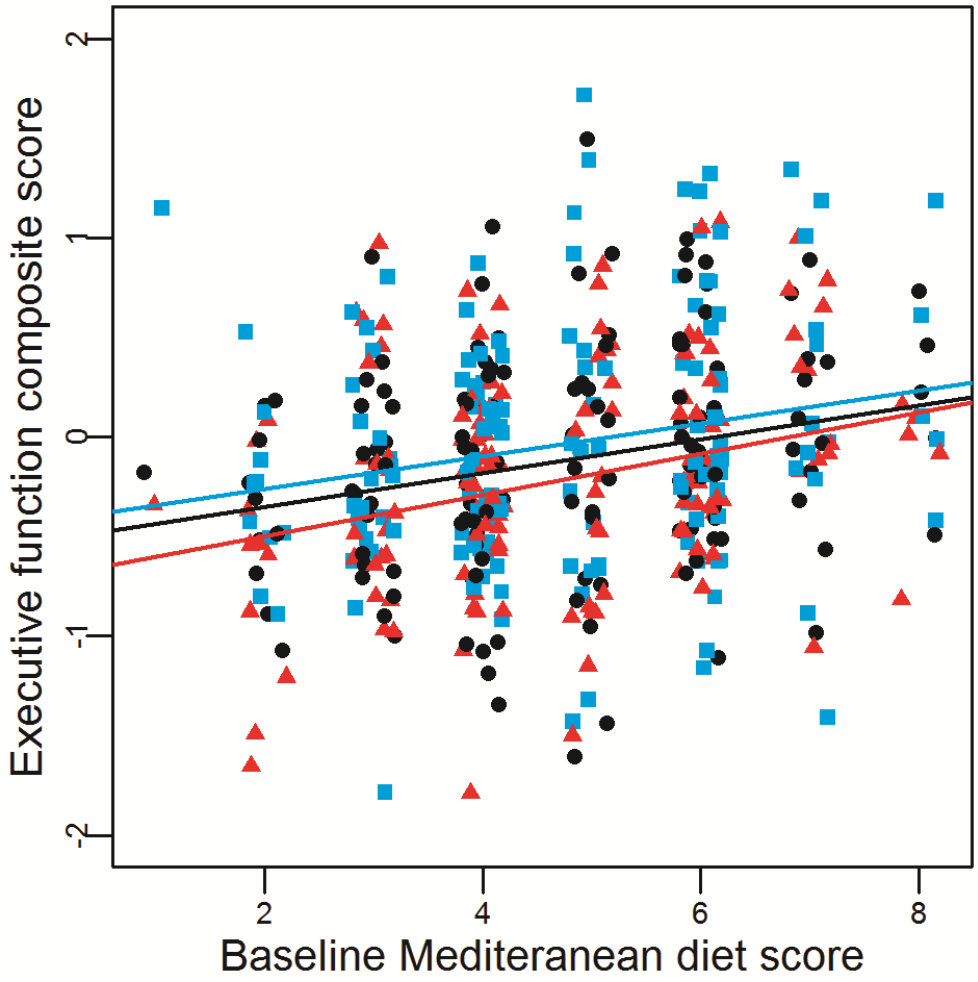

Figure 3.2: *Higher baseline MeDi adherence is associated with longitudinally increased performance in the executive function composite score in APOE ε4 allele carriers.*

Time 0 months; Time 18 months; Time 36 months.

Abbreviations: *APOE*, Apolipoprotein E; MeDi, Mediterranean diet.

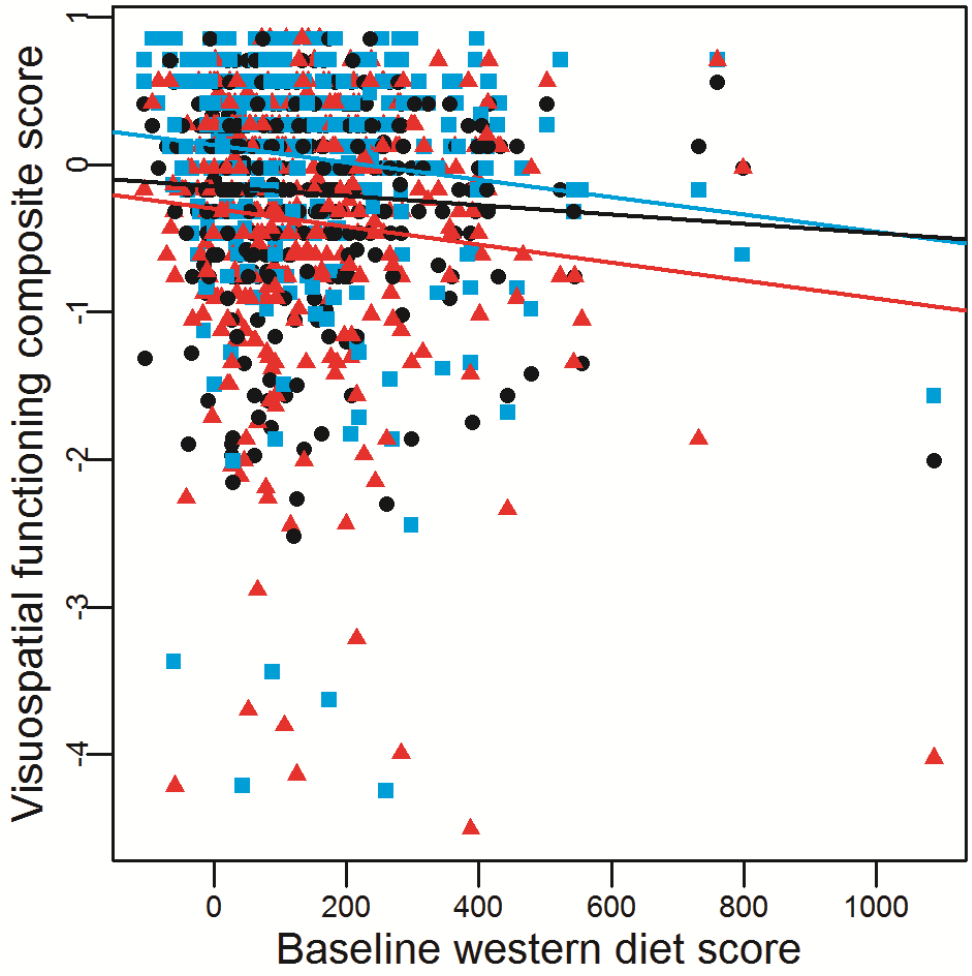

Figure 3.3: *Higher baseline western diet adherence is associated with longitudinally poorer performance in the visuospatial functioning composite score in APOE ε4 allele non-carriers.*

Time 0 months; Time 18 months; Time 36 months.

Abbreviations: *APOE*, Apolipoprotein E.

3.2.7 Dietary pattern adherence and transition from healthy control to mild cognitive impairment or Alzheimer's disease clinical classification

In order to assess whether baseline adherence to the four dietary patterns of interest was associated with change in clinical classification from healthy control to MCI or AD at 36 month follow-up, a change in clinical classification variable was made by assigning each participant a 0 if they remained a healthy control from baseline to the 36 month follow-up and a score of 1 if they transitioned to MCI or AD status. This change in clinical classification variable was then used in multinomial logistic regressions to calculate odds ratios for the investigation of the association between change from healthy control clinical classification to MCI or AD status and the four diet scores divided into tertiles; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The same two models utilised previously in this chapter were again used for the transition analysis; the initial model included the most common confounding variables as covariates, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, energy intake, smoking status, and history of hypertension, angina, stroke, diabetes and heart attack, and the second model incorporated the same confounding variables as the initial model without the inclusion of the vascular risk factors, i.e. smoking status, and history of hypertension, angina, stroke, diabetes and heart attack.

None of the four dietary patterns assessed were shown to be associated with the change in clinical classification variable; eating more 'unhealthy' or less anti-inflammatory foods did not lead to an increase in numbers transitioning from healthy control to MCI or AD status, and vice versa, i.e. eating more 'healthy' foods did not lead to a decrease in numbers transitioning from healthy control to MCI or AD status after 36 months. Furthermore, no significant associations were observed when the cohort was stratified by gender and *APOE* ε4 allele status (Table 3.13). It is worthy of note however, that

only 25 participants transitioned from healthy control at baseline to MCI or AD by the 36 month follow-up.

Table 3.13: *Odds Ratios of transitioning to AD or MCI clinical classification compared to remaining a healthy control after 36 months.*

Group+	Diet Score	Number of transitioners	Model	Odds Ratio	Confidence	*p*-value	Model	Odds Ratio	Confidence	*p*-value
Whole cohort	MeDi	25	1	0.889	0.400 – 1.980	0.773	2	0.817	0.374 – 1.790	0.612
Males	MeDi	15	1	0.772	0.281 – 2.120	0.616	2	0.678	0.254 – 1.810	0.439
Females	MeDi	10	1	0.748	0.141 – 3.960	0.733	2	0.797	0.176 – 3.600	0.768
APOE ε4 allele carrier	MeDi	13	1	0.661	0.252 – 1.730	0.398	2	0.702	0.315 – 1.570	0.388
APOE ε4 allele non - carrier	MeDi	12	1	0.857	0.269 – 2.729	0.794	2	0.923	0.308 – 2.769	0.886
Whole cohort	Prudent	25	1	0.835	0.470 – 1.480	0.538	2	0.824	0.463 – 1.470	0.511
Males	Prudent	15	1	0.805	0.390 – 1.660	0.559	2	0.808	0.391 – 1.670	0.565
Females	Prudent	10	1	0.718	0.224 – 2.300	0.577	2	0.767	0.238 – 2.470	0.656
APOE ε4 allele carrier	Prudent	13	1	0.575	0.195 – 1.690	0.316	2	0.719	0.292 – 1.770	0.474
APOE ε4 allele non - carrier	Prudent	12	1	0.790	0.379 – 1.647	0.781	2	0.901	0.423 – 1.919	0.530
Whole cohort	Western	25	1	0.932	0.550 – 1.580	0.795	2	0.939	0.556 – 1.590	0.813
Males	Western	15	1	0.929	0.447 – 1.930	0.844	2	0.960	0.463 – 1.990	0.912
Females	Western	10	1	1.042	0.365 – 2.980	0.939	2	0.975	0.349 – 2.720	0.961
APOE ε4 allele carrier	Western	13	1	0.736	0.262 – 2.060	0.560	2	0.726	0.301 – 1.750	0.475
APOE ε4 allele non – carrier	Western	12	1	1.119	0.562 – 2.228	0.748	2	1.081	0.559 – 2.094	0.816
Whole cohort	Inflammatory dietary index	25	1	1.296	0.841 – 1.996	0.240	2	1.280	0.839 – 1.953	0.252
Males	Inflammatory dietary index	15	1	1.097	0.585 – 2.056	0.773	2	1.473	0.784 – 2.769	0.229
Females	Inflammatory dietary index	10	1	1.426	0.751 – 2.706	0.278	2	1.085	0.999 – 1.001	0.788
APOE ε4 allele carrier	Inflammatory dietary index	13	1	1.555	0.813 – 2.974	0.182	2	1.622	0.870 – 3.024	0.128
APOE ε4 allele non – carrier	Inflammatory dietary index	12	1	0.971	0.525 – 1.797	0.925	2	0.946	0.524 – 1.714	0.860

Model 1^{+} - adjusted model includes age, gender, years of education, *APOE* ε4 allele carrier status, country of birth (Australia vs. Other), baseline body mass index, baseline energy intake kCal, past smoking status, and history of hypertension, angina, stroke, heart attack and diabetes as covariates. Model 2^{+} - adjusted model without CVD risk factors includes the same covariates as the fully adjusted model, without past smoking status, history of hypertension, angina, stroke, heart attack and diabetes. $^{+}$When stratified by gender and *APOE* ε4 allele status, these characteristics are not included in the models as covariates. Multinomial logistic regression analysis used to calculate odds ratios. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

3.3. Discussion

The objective of this analysis was to investigate the association of four dietary patterns; MeDi, inflammatory dietary index, western diet and prudent diet, firstly with baseline clinical classification, then with cognition and cognitive decline over a three year period amongst individuals classified as healthy controls at baseline, and finally transition from healthy control to MCI or AD clinical classification after three years.

3.3.1. Adherence to dietary patterns and baseline clinical classification

Our analysis suggests that AD participants in our cohort consume less foods in the MeDi and prudent diet, less anti-inflammatory foods, and more foods in the western diet than healthy controls. Healthy controls consume more foods in the MeDi and prudent diet, and more anti-inflammatory foods than MCI. There was no difference in western diet consumption between MCI and healthy control participants. MCI and AD participants consume the same amount of anti-inflammatory foods and MeDi foods, but MCI subjects consume more foods in the prudent diet and less foods in the western diet than AD subjects.

These results are cross-sectional, so we cannot assume that they show decreased adherence to the 'healthy' dietary patterns is a risk factor for AD, only that in our cohort at baseline, the AD participants had a decreased adherence to the 'healthy' dietary patterns, an increased adherence to the 'unhealthy' dietary pattern and a decreased consumption of anti-inflammatory foods. These results could potentially be explained by a change in diet due to AD diagnosis. To our knowledge, previously published research has concentrated on longitudinal risk of AD and MCI in the context of dietary patterns, rather than the association between cross-sectional clinical classification and diet. To exclude the potential confounder of diagnosis impacting on diet pattern adherence, for subsequent analysis individuals diagnosed at baseline as MCI or AD were excluded.

3.3.2. Correlation of the four dietary patterns

When assessing the correlation of the four dietary patterns, we observed that as prudent diet adherence increases, MeDi adherence increases, and western diet adherence decreases. The relationship between the MeDi and prudent diet scores is expected as both are 'healthy' eating patterns. The negative correlation between the prudent and

western dietary patterns is consistent with individuals eating less 'unhealthy' western diet foods if they are eating more 'healthy' prudent diet foods. We did not see a correlation between MeDi and western diet adherence, suggesting that the consumption of MeDi constituents is likely independent of consumption of western diet constituents, i.e. individuals can have a high MeDi score and a low, middle or high western diet score, or a low MeDi score and a low, middle or high western diet score. As anti-inflammatory food consumption increases (inflammatory dietary index decreases), adherence to MeDi and prudent dietary patterns increases, and somewhat counter-intuitively, western diet adherence also increases. The MeDi and prudent diets are rich in antioxidants such as fruit and vegetables which likely contribute to the correlation with the inflammatory dietary index. The association between increased consumption of anti-inflammatory foods and increased adherence to the western diet could in part be due to the high proportion of red and processed meat, and poultry consumed as part of the western diet: these components also contain some of the main constituents of the inflammatory dietary index, including niacin, thiamine, riboflavin, iron and zinc.

3.3.3. Adherence to dietary patterns and cognitive decline

When assessing the relationship between dietary patterns, cognition and cognitive decline amongst individuals classified as healthy control at baseline, we found that 1) there were no cross-sectional associations between the four dietary patterns and global cognition or the six composite cognitive domains assessed, 2) longitudinally, there were no gender-dependent associations between the four dietary patterns and global cognition or the six composite cognitive domains assessed. Thirdly, it was found that higher baseline adherence to the 'healthy' MeDi was associated with decreased decline in the executive function composite score 36 months later in *APOE* ε4 allele carriers only, and 4) higher baseline adherence to the 'unhealthy' western diet score was associated with increased cognitive decline in the visuospatial functioning composite score 36 months later, however in this case only in *APOE* ε4 allele non-carriers.

We saw no *APOE* genotype-dependent associations between adherence to any of the four dietary patterns and global cognitive score over 36 months, suggesting that the relationship between dietary pattern adherence and cognition is specific to the executive function and visuospatial functioning domains in our cohort.

A potential mechanism through which diet may exert its effect is via the vascular system (104, 284, 285). Scarmeas *et al*. (3) investigated whether there was attenuation of the association between MeDi and AD when vascular variables (stroke, diabetes mellitus, hypertension, heart disease, lipid levels) were simultaneously introduced into their logistic regression models: they reported that their observed association between MeDi and AD was not mediated by vascular comorbidities. To investigate the influence of vascular variables in our population, we analysed two adjusted models for the cross-sectional and longitudinal composite cognitive scores analysis; one controlling for vascular comorbidities and one without inclusion of these covariates. Although the addition of the CVD risk factors did not make any previously significant associations non-significant, or vice versa, we did see a change in the significance level of the executive function association with the MeDi in *APOE* ε4 allele carriers when the CVD risk factors were excluded ($p < 0.01$ to $p < 0.001$). However, the effect size was increased in the model without CVD risk factors, suggesting that, in agreement with Scarmeas *et al.* CVD risk factors do not appear to contribute significantly to the observed relationship between MeDi and cognition, and the observed improved significance may be due to lowering the degrees of freedom.

The deleterious effect of the western diet on visuospatial functioning was smaller in size than the association seen with the MeDi and executive function, and this association is likely driven by variation on the Rey Complex Figure Test (RCFT) copy as our clock test (the other test comprising the visuospatial functioning domain) data was negatively skewed and kurtosed. Within the RCFT copy there is an executive function domain, namely planning and use of strategy (268, 286). It could be that instead of a negative association between the western diet and visuospatial functioning domain, we are actually observing a negative association with the western diet in another area of executive function (planning), and therefore the only domain that diet is affecting is executive function. Of note, the measures comprising the executive function domain in the present study were heavily loaded with measures requiring cognitive flexibility, switching, and generativity, but did not include executive tasks relying heavily on planning. A recommendation for future studies is to measure more comprehensively the executive function components included in the visuospatial functioning domain to confirm our reasoning.

Worsening of executive function is often a debilitating feature of cognitive decline and AD, and has been shown to be related to decline in activities of daily living and to predict conversion from MCI to AD (287). Executive functions are a core component of self-control and self-regulation ability which has been shown to have broad and significant implications for everyday lives (288). Executive skills help us to connect past experiences with present actions, plan future behaviour when faced with novel tasks, judge, organise, change behaviour and strategies, pay attention and remember details for decision making (289).

We saw no associations with the inflammatory dietary index or the 'healthy' prudent dietary pattern and cognitive decline. The MeDi and prudent diet patterns are similar but have some significant differences, most notably characterisation of fish intake which contributes heavily to the MeDi but only represents a small component of the prudent diet score. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are n-3 fatty acids found in oily fish. These fatty acids are able to affect a number of aspects of inflammation, and mechanisms underlying their actions include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription factor nuclear factor kappa B so reducing expression of inflammatory genes, activation of the anti-inflammatory transcription NR1C3 and binding to the G protein-coupled receptor GPR120. EPA and DHA also increase anti-inflammatory and inflammation-resolving resolvins and protectins (290). Neuroinflammation is a process involved in AD pathogenesis which is thought to be triggered mostly by Aβ, a hallmark of AD pathology (291). It could be that the protective effect of MeDi adherence compared to the prudent diet is due to the consumption of fish oils such as EPA and DHA. Conversely, the negative association seen with the western diet and aspects of cognition may be due to low consumption of DHA and EPA for example, rather than high consumption of a detrimental food group. Several studies have provided reasons for this – for example, EPA and DHA fed to cells in culture increases production of sAPPα (known to promote neurite outgrowth), which may also indicate less Aβ will be produced (292). Furthermore, in studies of microglial cultures, both DHA and EPA were found to stimulate microglial phagocytosis of $A\beta_{1-42}$, and decrease levels of pro-inflammatory cytokines (293). However in some earlier studies, DHA and EPA were found to increase neuronal cell death following exposure to $A\beta_{1-42}$ (294), and some clinical studies involving dietary supplementation with DHA

have not demonstrated clear benefits in people with AD, however this may due to the fact that the lipids may only be beneficial at pre-clinical stages. It is clear that a better understanding of DHA (and EPA) metabolism and beneficial effects is required (295).

Previous studies have reported associations between dietary patterns and biomarkers of inflammation, for example the MeDi has been associated with lower levels of inflammatory markers (214, 227, 231, 296, 297), in other dietary pattern studies a 'healthy' dietary pattern (like our prudent diet) has been shown to lower inflammation marker levels and an 'unhealthy' dietary pattern (like our western diet) has been shown to increase levels of inflammatory markers (212, 298-302). Although the mechanism mediating the dietary effects cannot be discerned from the present results, the effect of MeDi constituents on inflammation is a prime candidate that warrants further investigation.

Our results suggest that there are differential effects of diet on cognition that are likely to be contingent on *APOE* ε4 allele carriage; another finding that warrants further investigation. Compared to the *APOE* ε2 and ε3 isoforms, *APOE* ε4 is a less functional cholesterol transporter (303). Management of cholesterol by fish oils in specific diets, like the MeDi, has been proposed to prevent and treat the negative effects of the *APOE* ε4 genotype (304). Animal studies have shown a positive influence of fish oil containing diets on behavioural and cognitive performances on *APOE* ε4 allele target replacement mice (305). A possible explanation for the MeDi effects observed only in *APOE* ε4 allele carriers in our study is that the non-carriers are already functioning highly in their cholesterol transport due to their ε2/ε3 genotype so MeDi components are not able to exert any additional significant effect. Ours is not the first study to report differential effects of lifestyle factors on aspects of cognitive decline and AD-related pathology that are contingent on *APOE* ε4 allele carriage. Notably, Brown *et al.* (132) showed that brain and blood Aβ levels can be modulated by physical activity, in an *APOE* genotype-dependent manner. Specifically, the authors reported that high physical activity is associated with lower plasma $A\beta_{1\text{-}42/1\text{-}40}$ in *APOE* ε4 allele non-carriers, while higher exercising *APOE* ε4 allele carriers had lower levels of brain amyloid. Brown *et al.* concluded that physical activity appears to attenuate the high levels of brain amyloid associated with *APOE* ε4 allele carriers, and the apparent lack of effect on plasma Aβ levels may reflect poor clearance of Aβ in the periphery in individuals with the *APOE*

ε4 allele. Taken together, the results of both studies suggest that in future, the assessment of lifestyle in the context of *APOE* genotype may help the development of strategies tailored to the needs of *APOE* ε4 allele carriers, both with and without AD, who have the poorest prognosis with current treatment strategies.

The positive relationship between MeDi adherence and better performance on the executive function composite score described in this paper is consistent with previous findings in other populations. In several American population studies, Scarmeas *et al*. reported higher MeDi adherence was associated with lower AD risk and slower cognitive decline (2-4). In a French study of 1410 individuals without dementia at baseline and with a follow-up within five years, it was observed that each additional MeDi score unit was associated with fewer Mini-Mental State Examination (MMSE) errors at follow-up (1). A study conducted in Chicago found that higher MeDi scores were associated with slower rates of cognitive decline in 3790 adults over the age of 65. Each participant had at least two cognitive assessments at three year intervals, which consisted of four cognitive tests (5). By contrast, the Personality and Total Health (PATH) through life study observed that greater MeDi adherence was not protective against cognitive decline. The apparent lack of protection in this study could be explained by the heterogeneous nature of the study population. Furthermore, only 66 participants from the original 1528 demonstrated any cognitive decline in the four year follow-up; a limitation with respect to generating sufficient statistical power to detect effects of the MeDi (226). Samieri *et al.* (282) found that long term MeDi adherence was related to moderately better cognition, but not with cognitive change in a large cohort of older women from the Nurses' Health Study (NHS). This study used a limited telephone interview to assess cognitive status, with only global cognition and verbal memory assessed. Another study by Samieri *et al.* (283), this time using the Women's Health Study (WHS), found no association with MeDi and cognitive decline. Again this study used the same telephone interview with only global and verbal memory assessed. Construction of the MeDi scores used in the NHS and WHS studies was modified from the 'traditional' method to increase relevance to American populations, for example refined and whole grains were delineated in the modified MeDi. The second *'a priori'* dietary pattern we assessed, the inflammatory dietary index, has not previously been analysed in a longitudinal prospective study of ageing cohort. Furthermore, to our

knowledge, the association of anti- or pro-inflammatory food consumption with cognitive decline has also not been assessed previously.

There are limited studies relating '*a posteriori*' dietary patterns such as the western and prudent patterns investigated here, to cognitive decline in the elderly. This could be due to identified patterns depending on the study cohort, thereby limiting inter-study comparison (243). Samieri *et al*. (6) identified five dietary patterns by cluster analysis, the 'healthy diet' pattern was associated with significantly lower MMSE errors. Akbaraly *et al*. (7) found that higher consumption of a 'whole food' pattern (similar to our prudent diet pattern), produced by principal components analysis, was associated with lower odds of cognitive deficit; whereas higher consumption of a 'processed food' pattern (similar to our western diet pattern) was associated with higher odds of cognitive deficit. However, adjustment for education significantly attenuated most of the associations seen.

3.3.4. Adherence to dietary patterns and transition from healthy control classification

Finally, we found that dietary pattern adherence had no effect on the rate of transitioning from healthy control classification at baseline to MCI or AD status after 36 months in the cohort as a whole and following stratification of the cohort by gender and *APOE* ε4 allele status. It should be noted however, that over the 36 month period of follow-up, only 25 of the 527 participants (4.5%) assessed at baseline transitioned from healthy control to MCI or AD status by the 36 month follow-up. This relatively small percentage of 'transitioners' may have provided insufficient statistical power to detect associations between the dietary patterns and change in clinical classification at follow-up; it is possible that when available, the 54 month follow-up clinical classifications will provide further insight.

3.3.5. Summary

In conclusion, our findings are consistent with previous studies which describe a 'healthy' (or 'prudent') dietary pattern in opposition to a 'western' (or 'processed') dietary pattern (6, 7, 219, 244, 245). To our knowledge, this is the first study extensively comparing the MeDi, inflammatory dietary index, western and prudent diet patterns to cognition and cognitive decline in an elderly, Australian cohort. Cross-sectionally, our analysis suggests that AD participants in our cohort consume fewer foods in the MeDi and prudent diet, less anti-inflammatory foods, and more foods in the western diet than healthy controls. Longitudinally, our results suggest a detrimental nature of a western diet and propose the importance of adhering to a 'healthy' dietary pattern such as the MeDi, with respect to reducing risk for cognitive decline, with executive function and visuospatial functioning seemingly most susceptible to the influence of diet. Additionally, dietary pattern adherence had no effect on the rate of transition from healthy control classification at baseline to MCI or AD status after 36 months, although limited statistical power likely impacts upon these findings. In the next chapter we will investigate the potential biological mechanisms underlying the relationships between diet and cognitive decline reported in this chapter.

Chapter Four

Investigating the mechanisms of action of dietary patterns

4.1. Introduction

In Chapter 3 we reported that higher baseline adherence to the 'healthy' Mediterranean diet (MeDi) was associated with decreased decline in the executive function cognitive domain composite score over 36 months in Apolipoprotein E (*APOE*) ε4 allele carriers. By contrast, higher baseline adherence to the 'unhealthy' western diet score was associated with increased decline in the visuospatial functioning cognitive domain composite score over 36 months in *APOE* ε4 allele non-carriers. In this chapter, we therefore sought to explore potential mechanisms that might underlie these observed effects of diet on cognition by assessing the relationship of the dietary patterns with blood and neuroimaging biomarker data collected as part of the Australian Imaging, Biomarkers and Lifestyle study (AIBL) of ageing. A plethora of potential mechanisms exist, we focussed our investigations on factors that are well-established contributors to Alzheimer's disease (AD) risk i.e. metabolic syndrome, cardiovascular disease (CVD), and inflammation.

Metabolic syndrome is a multifactorial disorder of energy utilization and storage, diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density lipoprotein (HDL) levels. Accumulating evidence suggests that metabolic syndrome is a risk factor for AD (306-309). The molecular mechanism underlying this relationship is not fully understood, however the cellular and biochemical alterations observed in metabolic syndrome, such as impairment of endothelial cell function, abnormality in essential fatty acid metabolism and alterations in lipid mediators along with abnormal insulin/leptin signalling, are likely contributors (307).

CVD refers to any disease or condition of the heart and blood vessels. The causes of CVD are diverse, but atherosclerosis and/or hypertension are the most common instigators. CVD and metabolic syndrome have many common risk factors, including elevated blood pressure, obesity, abnormal insulin metabolism and abnormal cholesterol levels. Data consistently suggests a strong and likely causal association between CVD, its risk factors, and AD incidence; individuals with subclinical CVD have been shown to be at greater risk of AD (75, 76, 104, 284, 310-313). Several CVD risk factors are also risk factors for AD, including hypertension, high low density lipoprotein (LDL) cholesterol, low HDL cholesterol and diabetes. The mechanisms underlying the CVD

and AD association are not fully elucidated, however, several candidate mechanisms have been proposed including; 1) both diseases share similar risk factors, which independently increase both CVD and AD occurrence; 2) vascular damage in the brain could create conditions which predispose to neurodegeneration; and 3) CVD risk factors could affect AD development directly by causing neuronal death, the accumulation of amyloid beta (Aβ), and the deposition of plaques and neurofibrillary tangles (312).

Abundant evidence suggests MeDi adherence reduces risk of metabolic syndrome and CVD, with MeDi's protective effect on obesity and type 2 diabetes proposed as the primary mechanism (225, 314-319). In the Attica study, MeDi adherence was associated with a 20% lower risk of having metabolic syndrome (320), and a meta-analysis investigating MeDi and health status concluded adherence to the MeDi significantly decreases risk of CVD, along with overall mortality and incidence of AD (225). In regards to 'healthy' versus 'unhealthy' dietary patterns (similar to the prudent and western diet patterns constructed in this thesis), 'healthy' pattern adherence has been found to decrease incidence of CVD risk factors and metabolic syndrome, whilst conversely, 'unhealthy' patterns have been shown to increase the relative risk of these disorders (321-323). For example, a review found dietary patterns high in fruit and vegetables (similar to our prudent diet pattern) were associated with lower prevalence of metabolic syndrome, and those high in meat intake (similar to our western diet pattern) were associated with components of metabolic syndrome (322).

Inflammation is heavily implicated as an important component of AD, with evidence suggesting that it is both a reaction to the disease process and a contributor to neuronal damage (324-326). Recent genetic studies provide further evidence that inflammatory processes are important in the pathogenesis of AD (327-330), for example Jonsson *et al.* (327) found a rare missense mutation in the gene encoding triggering receptor expressed on myeloid cells 2 (TREM2) conferred significantly increased risk of AD; TREM2 has an anti-inflammatory role in the brain, and the TREM2 variant (an R47H substitution) may lead to an increased predisposition to AD through impaired containment of inflammatory processes. The inflammation process involves the production of inflammatory molecules including cortisol, C-reactive protein (CRP) and interleukins (IL), many of which have been associated with dietary patterns. For example higher MeDi adherence has been associated with lower CRP, IL6, IL7, and IL18 levels, and lower white cell count compared with individuals with low MeDi adherence (214, 227,

231, 296, 297). Reported results have however, been somewhat inconsistent, for example, Dai *et al*. (331) found higher adherence to the MeDi was associated with reduced levels of IL6 but had no significant effect on CRP, whilst an intervention study where participants consumed an alcohol free MeDi or a high fat diet for 90 days reported no detectable effect in CRP levels (332). By contrast, Chrysohoou *et al*. (214) found an association between MeDi adherence, CRP, IL6, and white cell count, but found no association with tumour necrosis factor alpha (TNFα) levels. In regards to other dietary patterns, a 'healthy' dietary pattern (similar to our prudent diet pattern) has been shown to be inversely related to concentrations of CRP, IL6 and vascular cell adhesion molecule 1 (299, 300), whilst an 'unhealthy' dietary pattern (similar to our western diet pattern) has been positively associated with serum amyloid A, IL6, and CRP levels (212, 299).

In addition to assessing the relationship between the four dietary patterns described in Chapter 3 and the wealth of blood-based analytes measured as part of the AIBL study, this chapter will also report on the relationship between the four dietary patterns and neuroimaging biomarkers: specifically Pittsburgh Compound-B positron emission tomography (PiB PET)-determined cerebral amyloid load (a hallmark of AD), and magnetic resonance imaging (MRI)-determined brain volume. Whilst higher MeDi adherence has been associated with reduced odds of having a cerebral infarct (a small localized area of dead tissue resulting from blood supply failure; 235) as visualised using MRI, other studies have concentrated on how individual dietary constituents affect brain volumes and atrophy (97, 333, 334); for example, the meat component of the MeDi score has been negatively associated with summed grey and white matter volume, but not the MeDi score as a whole (335). However, studies reporting on dietary pattern adherence and neuroimaging biomarkers are few in number and, to our knowledge, there are no studies to date investigating the relationship between diet and brain amyloid load.

4.1.1. Methods

We assessed the relationship of the four dietary patterns described in Chapter 3 (MeDi, prudent, western and inflammatory dietary index) with blood-based biomarkers of metabolic syndrome, CVD and inflammation as well as with neuroimaging biomarkers of AD. Our aims were investigated in the cohort utilised in the longitudinal cognition analysis reported in Chapter 3, i.e. a well characterised, elderly, Australian healthy control cohort, taken from the larger AIBL study (11). Keeping methods consistent with the longitudinal cognitive data reported in Chapter 3, which has been published in Molecular Psychiatry, a *p*-value of 0.01 or smaller determined a significant result for all analyses to balance the risk of type I and type II errors.

4.1.2. Chapter aims

This chapter utilises dietary data, and blood and brain biomarker data collected as part of the longitudinal prospective AIBL study to address the following aims:

1. To explore the potential mechanism that might underlie the observed effects of dietary patterns on cognitive decline reported in Chapter 3 through investigating the relationship of the dietary patterns with blood-based biomarkers of metabolic syndrome, CVD and inflammation both cross-sectionally and longitudinally over 36 months.

2. To investigate whether the dietary patterns are associated with brain region volumes, (measured by MRI), and cerebral amyloid burden (measured by PiB PET), or longitudinal change in these variables over 36 months.

Whilst the existing literature is limited, we hypothesise that:

1. Higher adherence to the 'healthy' MeDi or prudent dietary patterns, lower adherence to the 'unhealthy' western dietary pattern or increased consumption of anti-inflammatory foods (lower inflammatory dietary index) is associated with decreases in levels of CVD and metabolic syndrome blood-based biomarkers.

2. Higher adherence to the 'healthy' MeDi or prudent dietary patterns, lower adherence to the 'unhealthy' western dietary pattern or increased consumption of anti-inflammatory foods is associated with decreases in levels of blood-based biomarkers of inflammation.

3. Higher adherence to the 'healthy' MeDi or prudent dietary patterns, lower adherence to the 'unhealthy' western dietary pattern or increased consumption of anti-inflammatory foods at baseline is associated cross-sectionally with increased brain volume and longitudinally with decreased cortical and hippocampal atrophy.

4. Higher adherence to the 'healthy' MeDi or prudent dietary patterns, lower adherence to the 'unhealthy' western dietary pattern or increased consumption of anti-inflammatory foods at baseline is associated cross-sectionally with lower cerebral amyloid burden and longitudinally with slower accumulation of cerebral amyloid.

4.2. Results

4.2.1. Descriptive statistics for the healthy control cohort

This analysis included all healthy control participants with a completed Cancer Council of Victoria food frequency questionnaire (CCVFFQ) at baseline that were included in the longitudinal cognition analysis in Chapter 3.

The demographics of this cohort and comparison between genders and *APOE* ε4 allele carriers and non-carriers were described in section 3.2.3 and Table 3.4. Briefly, the main differences between males and females were a higher percentage of females born in Australia, a higher percentage of females with 12 or less years of education, and females had a lower mean body mass index (BMI). Males had a higher energy intake, and a higher percentage of people with a history of angina, heart attack and who were past smokers. With respect to the diet scores, western diet score was significantly higher in males and inflammatory dietary index was significantly higher in females. The only difference between *APOE* ε4 allele carriers and non-carriers was a higher prudent diet score in non-carriers.

A subgroup of the cohort utilised for this analysis underwent brain imaging. Participants were selected for the neuroimaging study based on their willingness to participate in neuroimaging (as indicated on the consent form) and their eligibility for neuroimaging (e.g. individuals with contraindicative metal objects or claustrophobia were excluded from MRI). The demographics of the neuroimaging sub-cohort are described in section 4.2.5.1. and Table 4.11.

4.2.2. Diet scores and metabolic syndrome

Metabolic syndrome was the first mechanism investigated as a possible explanation of the cognition and dietary pattern results observed in Chapter 3. A metabolic syndrome index was constructed for each individual. Participants were given one point for each of the following five components; 1) obesity (BMI ≥ 25); 2) raised blood pressure (systolic ≥ 140 and/or diastolic ≥ 90 and/or a history of hypertension); 3) high triglyceride level (> 2 mmol/L); 4) low high density lipoprotein level (≤ 1.0 mmol/L) and; 5) impaired fasting glucose and/or a history of diabetes (glucose level 6.1 ≥ x ≤ 6.9 mmol/L). These components were utilised as they are the most common factors defining metabolic syndrome (336): they are clinical parameters with clinically determined ranges which were used to construct the index and subsequently define high and low risk of metabolic syndrome. The points were summed to produce an index ranging from 0 - 5, with a higher score indicating higher risk of metabolic syndrome. Multiple linear regression analyses were run to determine the association between the continuous diet scores (independent variable) and the metabolic syndrome index, as well as the components of the index individually, and additional biomarkers of metabolic syndrome measured as part of the AIBL study (dependent variables). The most common confounding variables were controlled for in all analyses, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, and energy intake: these variables can affect the outcome of the analysis and failure to include them in the models can cause misestimates of relationships. As BMI is an element of the metabolic syndrome index, it was not included as a covariate in the analysis of the index or when the components of the index were analysed separately. Following stratification of the cohort based on gender and *APOE* ε4 allele carriage, all of these analyses were re-run.

There were no associations between the four baseline dietary scores and the metabolic syndrome index, or the individual components of the index in the cohort as a whole or when stratified by gender or *APOE* ε4 allele carriage. In regards to the additional biomarkers of metabolic syndrome measured as part of the AIBL study, insulin levels were negatively associated with prudent diet ($\beta = -0.120$, $p = 0.008$) in the cohort as a whole, with diet explaining 6.2% of the variance in this relationship (Table 4.1). Insulin was additionally positively associated with the inflammatory dietary index in the cohort as a whole ($\beta = 0.200$, $p < 0.001$; Table 4.1), as well as in females ($\beta = 0.238$, $p = 0.002$; Table 4.2) and *APOE* ε4 allele non-carriers ($\beta = 0.185$, $p = 0.004$; Table 4.3); the

inflammatory dietary index explained 2.8%, 3.5% and 7.8% of the variance in these relationships, respectively. Thus, a higher prudent diet score and a higher consumption of anti-inflammatory foods (indicated by a lower inflammatory dietary index) are both associated with decreased insulin levels.

Glucagon (a hormone secreted by the pancreas which stimulates increases in blood sugar levels, thus opposing the action of insulin) was negatively associated with western diet score in males ($\beta = -0.218$, $p = 0.007$; Table 4.2) and *APOE* ε4 allele non-carriers ($\beta = -0.207$, $p < 0.001$; Table 4.3), with diet explaining 13.3% and 9.4% of the variance in the relationships respectively. Glucagon-like peptide-1 (which reduces postprandial glycaemia by modulating gastric emptying and endocrine pancreatic secretion) was also negatively associated with western diet score though in *APOE* ε4 allele non-carriers only: and diet explained a 7.2% portion of the variance observed ($\beta = -0.175$, $p = 0.006$; Table 4.3).

A metabolic syndrome index was also constructed for 36 month follow up data, using the same method as that utilised during construction of the baseline index. The 36 month follow up index was subtracted from the baseline index for each participant to give a value for 'change in the index' over 36 months. If the index increased over 36 months participants were assigned a value of '1', if there was no change, participants were assigned a value of '2', and if the index decreased over 36 months, participants were assigned a value of '3'. This 'change in index' variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary adherence and change in metabolic syndrome risk over 36 months ('no change' i.e. a score of '2', was the reference). The four diet scores were divided into tertiles for this analysis; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, individuals with a score of 4 or 5 were assigned to tertile 2, whilst individuals with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The model was adjusted for the same potential confounders as those incorporated into the linear regression models described earlier in

this section. Dietary pattern adherence did not affect the likelihood of the metabolic syndrome index decreasing or increasing over 36 months, in the cohort as a whole or following stratification of the cohort by gender and *APOE* ε4 allele carriage (data not shown).

Table 4.1: *Relationship between baseline diet scores and metabolic syndrome biomarkers: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score	Baseline prudent diet score	Baseline western diet score	Baseline inflammatory dietary index
Metabolic syndrome index[a]	0.030	-0.052	0.041	0.120
Obesity[ab]	-0.020	-0.028	0.054	0.076
Raised blood pressure[b]	0.005	-0.041	0.025	-0.026
High triglyceride level[b]	-0.020	-0.083	0.036	0.064
Low high density lipoprotein level[b]	0.075	0.098	-0.113	-0.095
Impaired fasting glucose and/or a history of diabetes[b]	-0.010	0.015	-0.011	0.042
Adiponectin[cd]	0.055	0.005	0.035	-0.058
Angiotensinogen[ce]	0.018	0.102	-0.075	-0.077
Glucagon-like peptide-1[cf]	-0.041	0.044	-0.100	-0.008
Glucagon[cg]	-0.035	0.085	-0.129	0.002
Insulin[ch]	-0.073	**-0.120* (6.2%[t])**	-0.024	**0.200** (2.8%[t])**
Leptin[ci]	-0.061	-0.006	-0.049	0.025
Resistin[cj]	0.022	-0.066	0.028	0.120
ApoE protein[k]	0.023	0.010	-0.047	0.048

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the metabolic syndrome index analysed separately, the four other components of the index are also included as covariates. Bold indicates statistical significance (*$p < 0.01$, **$p < 0.001$). [t]Portion of variance explained by diet score. [a]Obesity and metabolic syndrome index do not include body mass index as a covariate. [b]Components of the metabolic syndrome index analysed separately. [c]Measured as part of Rules Based Medicine biomarker panel analysis. [d]A protein hormone that modulates a number of metabolic processes including glucose regulation and fatty acid catabolism. [e]Causes blood vessels to constrict, and drives blood pressure up. [f]Reduces postprandial glycaemia by modulating gastric emptying and endocrine pancreatic secretion. [g]A hormone secreted by the pancreas, which stimulates increases in blood sugar levels; thus opposing the action of insulin. [h]A hormone which has extensive effects on metabolism and other body functions such as vascular compliance. [i]A hormone that helps regulate energy intake and energy expenditure, including appetite and metabolism. [j]A protein suggested to be linked to obesity and type 2 diabetes. [k]Measured by commercial kit. Abbreviations: *APOE*, Apolipoprotein E gene; ApoE, Apolipoprotein E protein; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.2: *Relationship between baseline diet scores and metabolic syndrome biomarkers following stratification of the cohort by gender: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
Gender / **Biomarker**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Metabolic syndrome index[a]	0.028	-0.001	-0.089	-0.037	0.018	0.093	0.171	0.106
Obesity[ab]	0.010	-0.029	-0.042	-0.014	0.015	0.090	0.195	-0.026
Raised blood pressure[b]	-0.033	0.011	-0.131	0.029	0.090	-0.021	0.037	-0.071
High triglyceride level[b]	0.073	-0.084	-0.154	-0.019	0.061	-0.006	0.042	0.084
Low high density lipoprotein level[b]	0.083	0.058	0.161	-0.003	-0.165	0.031	-0.168	0.036
Impaired fasting glucose and/or a history of diabetes[b]	-0.093	0.051	0.045	0.003	-0.036	0.013	-0.047	0.129
Adiponectin[cd]	0.020	0.112	-0.037	0.065	0.069	0.007	-0.012	-0.158
Angiotensinogen[ce]	-0.071	0.093	0.114	0.096	-0.087	-0.049	-0.074	-0.093
Glucagon-like peptide-1[cf]	-0.063	-0.018	0.108	-0.024	-0.187	0.044	0.038	-0.097
Glucagon[cg]	-0.088	0.019	0.163	-0.017	**-0.218* (13.3%[i])**	0.049	0.095	-0.152
Insulin[ch]	-0.026	-0.124	-0.086	-0.158	-0.067	0.032	0.157	**.38* (3.5%[i])**
Leptin[ci]	-0.015	-0.083	-0.059	-0.021	-0.052	-0.055	0.114	0.055
Resistin[cj]	-0.032	0.090	-0.181	0.070	0.080	-0.063	0.208	0.019
ApoE protein[k]	0.027	0.024	0.016	0.013	-0.031	-0.064	0.049	0.045

Model includes age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the metabolic syndrome index analysed separately, the four other components of the index are also included as covariates. Bold indicates statistical significance (*$p < 0.01$). [i]Portion of variance explained by diet score. [a]Obesity and metabolic syndrome index do not include body mass index as a covariate. [b]Components of the metabolic syndrome index analysed separately. [c]Measured as part of Rules Based Medicine biomarker panel analysis. [d]A protein hormone that modulates a number of metabolic processes including glucose regulation and fatty acid catabolism. [e]Causes blood vessels to constrict, and drives blood pressure up. [f]Reduces postprandial glycaemia by modulating gastric emptying and endocrine pancreatic secretion. [g]A hormone secreted by the pancreas, which stimulates increases in blood sugar levels; thus opposing the action of insulin. [h]A hormone which has extensive effects on metabolism and other body functions such as vascular compliance. [i]A hormone that helps regulate energy intake and energy expenditure, including appetite and metabolism. [j]A protein suggested to be linked to obesity and type 2 diabetes. [k]Measured by commercial kit. Abbreviations: *APOE*, Apolipoprotein E gene; ApoE, Apolipoprotein E protein; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.3: *Relationship between baseline diet scores and metabolic syndrome biomarkers following stratification of the cohort by APOE ε4 allele carriage: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
***APOE* ε4 allele carrier status[L]**	-	+	-	+	-	+	-	+
Biomarker								
Metabolic syndrome index[a]	-0.017	0.037	-0.060	-0.063	0.064	0.005	0.117	0.149
Obesity[ab]	0.011	-0.160	-0.003	-0.118	0.059	0.059	0.048	0.158
Raised blood pressure[b]	-0.003	-0.003	-0.055	0.008	0.045	-0.038	0.009	-0.102
High triglyceride level[b]	-0.026	0.054	-0.127	0.089	0.078	-0.133	0.108	-0.082
Low high density lipoprotein level[b]	0.031	0.201	0.121	0.037	-0.133	-0.079	-0.067	-0.193
Impaired fasting glucose and/or a history of diabetes[b]	-0.039	0.104	0.007	0.020	0.000	0.022	0.059	-0.001
Adiponectin[cd]	0.054	0.073	0.003	0.002	0.047	0.029	-0.062	-0.002
Angiotensinogen[ce]	-0.006	0.062	0.128	0.006	-0.087	0.005	-0.102	0.030
Glucagon-like peptide-1[cf]	-0.024	-0.104	0.095	-0.085	**-0.175* (7.2%[l])**	0.116	-0.093	0.201
Glucagon[cg]	-0.008	-0.122	0.128	-0.029	**-0.207** (9.4%[l])**	0.102	-0.082	0.217
Insulin[ch]	-0.074	-0.097	-0.132	-0.068	-0.017	-0.063	**0.185* (7.8%[l])**	0.188
Leptin[ci]	-0.034	-0.095	-0.010	0.014	-0.028	-0.081	0.018	0.033
Resistin[cj]	0.054	-0.070	-0.063	-0.068	0.042	-0.041	0.095	0.170
ApoE protein[k]	0.032	0.069	0.037	-0.046	-0.097	0.158	0.062	-0.001

[L]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the metabolic syndrome index analysed separately, the four other components of the index are also included as covariates. Bold indicates statistical significance (*$p < 0.01$, **$p < 0.001$). [l]Portion of variance explained by diet score. [a]Obesity and metabolic syndrome index do not include body mass index as a covariate. [b]Components of the metabolic syndrome index analysed separately. [c]Measured as part of Rules Based Medicine biomarker panel analysis. [d]A protein hormone that modulates a number of metabolic processes including glucose regulation and fatty acid catabolism. [e]Causes blood vessels to constrict, and drives blood pressure up. [f]Reduces postprandial glycaemia by modulating gastric emptying and endocrine pancreatic secretion. [g]A hormone secreted by the pancreas, which stimulates increases in blood sugar levels; thus opposing the action of insulin. [h]A hormone which has extensive effects on metabolism and other body functions such as vascular compliance. [i]A hormone that helps regulate energy intake and energy expenditure, including appetite and metabolism. [j]A protein suggested to be linked to obesity and type 2 diabetes. [k]Measured by commercial kit. Abbreviations: *APOE*, Apolipoprotein E gene; ApoE, Apolipoprotein E protein; kCal, kilocalorie; MeDi, Mediterranean diet.

4.2.3. Diet scores and cardiovascular disease

Cardiovascular disease (CVD) was the second mechanism investigated as a possible explanation of the cognition and dietary pattern results observed in Chapter 3. A CVD index was constructed for each individual. Participants were given one point for each of the following nine components; 1) obesity (BMI ≥ 25); 2) raised blood pressure (systolic ≥ 140 and/or diastolic ≥ 90 and/or a history of hypertension); 3) a history of angina; 4) a history of stroke; 5) a history of heart attack; 6) a history of smoking; 7) a history of diabetes; 8) low physical activity (being in the lowest tertile of physical activity as measured by the International Physical Activity Questionnaire; IPAQ) and; 9) elevated homocysteine level (> 10 μmol/L). Analogous to the metabolic syndrome index construction, these components were utilised in the CVD index as they are the main risk factors for CVD (337). Obesity, raised blood pressure and homocysteine level are clinical parameters and therefore we utilised the clinically defined 'cut-offs'. The other components, excepting physical activity, are all categorical variables with a 'yes' or 'no' answer, whilst low physical activity was determined based on the cohort's reported activity levels. The points assigned to each individual were summed to produce an index ranging from 0 - 9, with a higher score indicating higher risk of CVD. There is considerable overlap between risk factors for metabolic syndrome and CVD, including obesity, elevated blood pressure, diabetes, increased LDL and decreased HDL: Several of these factors were used in both the metabolic syndrome and CVD indexes. Additional lipid profile data (including LDL and HDL subfractions) measured as part of the AIBL study was only included in this section which investigates the association of dietary patterns and CVD biomarkers.

The relationship between the four dietary patterns and levels of total cholesterol, triglycerides, HDL, and LDL was determined using the results of clinical tests undertaken by PathWest laboratories: This analysis was conducted cross-sectionally using baseline data, and longitudinally by subtracting the baseline level from the 36 month follow up level for each participant to yield a value for 'change in analyte levels' over 36 months. To supplement the PathWest lipid profile data, a Lipoprint system was used to divide the baseline lipid profile components further into large, small and intermediate HDL, very low

density lipoproteins (VLDL), intermediate density lipoprotein (IDL) subfractions (A, B and C), and LDL subfractions (1 to 2, and 3 to 7).

Multiple linear regression analyses were run to determine the association between the diet scores (independent variable), the CVD index, individual components of the index, the PathWest lipid profiles and the Lipoprint-determined lipid subfractions (dependent variables). As with the metabolic syndrome analysis in section 4.2.2., the most common confounding variables were controlled for in all analyses, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, and energy intake. As BMI is an element of the CVD index, it was not included as a covariate in the analysis of the index or when the components of the index were analysed separately. Following stratification of the cohort based on gender and *APOE* ε4 allele carriage, all analyses were re-run.

The index and its individual components were not associated with the four dietary patterns in the cohort as a whole (Table 4.4), or following stratification of the cohort by gender or *APOE* ε4 allele carriage (Tables 4.5 and 4.6).

Several significant results were observed with respect to the PathWest lipid profiles and the Lipoprint-determined lipid subfractions. Prudent diet score was negatively associated with triglyceride level in males (β = -0.203, $p < 0.006$; Table 4.5), and *APOE* ε4 allele non-carriers (β = -0.148, $p < 0.006$; Table 4.6), i.e. as prudent diet adherence increased, triglyceride levels decreased, with diet explaining 3.4% and 1.8% of the variance respectively. Western diet score was positively associated with IDL-C level in females (β = 0.193, $p = 0.003$; Table 4.5), i.e. as western diet adherence increased, IDL-C level also increased, with diet explaining 6.5% of the variance in this relationship. There were no associations between the lipid profiles and MeDi score or the inflammatory dietary index. Longitudinally however, there were no associations between change in the lipid profiles over 36 months and any of the four diet scores in the cohort as a whole or following stratification of the cohort by gender or *APOE* ε4 allele carriage (Tables 4.4 to 4.6).

A CVD index was also constructed for 36 month follow up data, using the same method as that utilised during construction of the baseline index. As per the longitudinal metabolic syndrome index analysis, the 36 month follow up CVD index was subtracted from the

baseline CVD index for each participant to give a value for 'change in the index' over 36 months. If the index increased over 36 months participants were assigned a value of '1', if there was no change participants were assigned a value of '2', and if the index decreased over 36 months, participants were assigned a value of '3'.

This 'change in index' variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary adherence and change in CVD risk over 36 months ('no change' i.e. a score of '2', was the reference). The four diet scores were divided into tertiles for this analysis, as described in section 4.2.2.; odds ratios compared the lowest tertile with the highest tertile. The model was adjusted for the same potential confounders as those incorporated into the linear regression models described earlier in this section.

Prudent diet adherence affected the likelihood of the CVD index remaining stable over 36 months. In females, the odds of the CVD index remaining at the baseline level rather than increasing at 36 months was 3.66 times higher in the highest tertile of prudent diet adherence compared to the lowest adherence tertile (Confidence interval (CI): 1.368 – 9.776; $p < 0.010$). In *APOE* ε4 allele carriers, the odds of the CVD index remaining at the baseline level rather than increasing at 36 months were also 7.03 times higher in the highest tertile of prudent diet adherence compared to the lowest adherence tertile (CI: 1.598 – 30.910: $p < 0.010$). These results are shown in Table 4.7.

Table 4.4: *Relationship between baseline diet scores and CVD biomarkers: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score	Baseline prudent diet score	Baseline western diet score	Baseline inflammatory dietary index
Cardiovascular disease index[a]	0.016	-0.069	0.058	0.074
Obesity[ia]	-0.013	-0.013	0.020	0.079
Low physical activity[ia]	-0.014	-0.071	-0.060	0.095
Raised blood pressure[ia]	0.014	-0.023	0.008	0.003
History of angina[ia]	-0.015	0.123	0.063	0.032
History of heart attack[ia]	0.021	-0.042	0.026	-0.018
History of stroke[ia]	-0.002	0.049	-0.049	-0.111
History of diabetes[ia]	0.037	0.004	-0.003	0.023
History of smoking[ia]	-0.035	0.010	0.057	-0.008
Elevated homocysteine level[ia]	0.065	-0.025	0.063	-0.024
Total cholesterol[b]	-0.019	-0.104	0.097	0.015
Triglycerides[b]	0.004	-0.114	0.051	0.079
High density lipoprotein[b]	-0.013	-0.034	0.069	0.015
Low density lipoprotein[b]	-0.018	-0.049	0.062	-0.014
High density lipoprotein large[c]	0.016	0.008	0.028	-0.002
High density lipoprotein intermediate[c]	-0.077	-0.073	0.100	0.035
High density lipoprotein small[c]	0.003	-0.100	0.100	0.069
Very low density lipoprotein[c]	-0.017	-0.078	0.022	0.075
Intermediate density lipoprotein A[c]	0.004	0.008	0.043	-0.042
Intermediate density lipoprotein B[c]	-0.003	-0.063	0.098	0.040
Intermediate density lipoprotein C[c]	-0.006	-0.104	0.106	0.037
Low density lipoproteins 1 and 2[c]	-0.004	-0.058	0.046	-0.001
Low density lipoproteins 3 – 7[c]	0.033	-0.027	0.043	0.069
Change in total cholesterol[b]	-0.024	0.026	-0.021	-0.013
Change in triglycerides[b]	-0.050	-0.060	0.095	0.000
Change in high density lipoprotein[b]	0.016	0.002	-0.041	-0.013
Change in low density lipoprotein[b]	-0.023	0.034	-0.023	-0.006

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the CVD index analysed separately, the eight other components of the index are also included as covariates. [i]Components of the CVD index analysed separately. [a]CVD index and individual components do not include body mass index as a covariate. [b]Measured by PathWest clinical pathology tests. [c]Baseline lipid subfractions determined using the Lipoprint system. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.5: *Relationship between baseline diet scores and CVD biomarkers following stratification of the cohort by gender: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline Inflammatory dietary index	
Gender Biomarker	Male	Female	Male	Female	Male	Female	Male	Female
Cardiovascular disease index[a]	0.008	0.016	-0.111	-0.030	0.044	0.065	0.132	0.037
Obesity[ia]	0.001	-0.014	-0.008	0.017	-0.024	0.055	0.185	-0.048
Low physical activity[ia]	-0.042	-0.009	-0.205	0.031	-0.020	-0.088	0.224	0.014
Raised blood pressure[ia]	-0.032	0.029	-0.117	0.016	0.047	0.018	0.067	-0.025
History of angina[ia]	-0.044	0.037	0.038	-0.014	0.008	0.065	-0.036	0.051
History of heart attack[ia]	0.111	-0.083	-0.048	-0.096	0.038	0.051	-0.030	0.044
History of stroke[ia]	0.023	-0.089	0.070	0.014	-0.051	-0.035	-0.110	-0.053
History of diabetes[ia]	0.053	0.063	0.058	0.006	-0.040	0.033	-0.059	0.069
History of smoking[ia]	-0.081	0.013	0.096	-0.046	0.025	0.063	-0.047	0.031
Elevated homocysteine level[ia]	0.099	0.004	-0.037	-0.057	0.123	-0.007	-0.060	0.060
Total cholesterol[b]	-0.003	-0.006	-0.131	-0.105	0.036	0.153	0.053	-0.029
Triglycerides[b]	0.036	0.005	**-0.203* (3.4%[†])**	-0.033	0.029	0.068	0.111	0.042
High density lipoprotein[b]	0.043	-0.042	-0.034	-0.033	0.148	0.019	0.002	-0.003
Low density lipoprotein[b]	-0.033	0.010	-0.064	-0.094	-0.031	0.144	0.021	-0.042
High density lipoprotein large[c]	0.045	0.011	0.019	0.025	0.085	-0.009	-0.066	0.009
High density lipoprotein intermediate[c]	0.001	0.129	-0.050	-0.093	0.180	0.046	0.044	-0.001
High density lipoprotein small[c]	0.026	-0.014	-0.098	-0.096	0.105	0.063	0.151	0.005
Very low density lipoprotein[c]	0.004	-0.038	-0.136	-0.029	-0.004	0.019	0.137	0.032
Intermediate density lipoprotein A[c]	0.005	0.014	0.046	-0.030	-0.001	0.100	-0.001	-0.083
Intermediate density lipoprotein B[c]	-0.051	0.034	-0.102	-0.055	0.073	0.124	0.119	-0.007
Intermediate density lipoprotein C[c]	0.004	-0.008	-0.120	-0.101	0.000	**0.193* (6.5%[†])**	0.114	-0.023
Low density lipoproteins 1 and 2[c]	-0.030	0.022	-0.050	-0.066	0.013	0.077	-0.091	0.077
Low density lipoproteins 3 – 7[c]	0.105	-0.027	-0.067	-0.003	0.093	-0.014	0.055	0.098
Change in total cholesterol[b]	-0.033	-0.015	-0.010	0.092	0.023	-0.091	0.017	-0.054
Change in triglycerides[b]	-0.033	-0.083	-0.082	-0.016	0.153	-0.009	0.000	-0.007
Change in high density lipoprotein[b]	0.054	-0.005	-0.016	0.018	-0.134	0.033	0.076	-0.066
Change in low density lipoprotein[b]	-0.051	0.006	-0.009	0.107	0.045	-0.117	0.008	-0.039

Model includes age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the CVD index analysed separately, the eight other components of the index are also included as covariates. Bold indicates statistical significance (*$p < 0.01$). [i]Components of the CVD index analysed separately. [†]Portion of variance explained by diet score. [a]CVD index and individual components do not include body mass index as a covariate. [b]Measured by PathWest clinical pathology tests. [c]Baseline lipid subfractions determined using the Lipoprint system. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.6: *Relationship between baseline diet scores and CVD biomarkers following stratification of the cohort by APOE ε4 allele carriage: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
***APOE* ε4 allele carrier status**[d] **Biomarker**	-	+	-	+	-	+	-	+
Cardiovascular disease index[a]	0.031	-0.031	-0.091	-0.020	0.072	0.047	0.063	0.110
Obesity[ia]	-0.005	-0.115	0.001	-0.155	0.022	0.102	0.039	0.239
Low physical activity[ia]	0.016	-0.087	-0.073	-0.054	-0.064	-0.108	0.076	0.205
Raised blood pressure[ia]	0.010	0.078	-0.056	0.072	0.040	-0.088	0.037	-0.053
History of angina[ia]	0.025	-0.081	-0.011	-0.041	-0.017	0.206	0.026	-0.087
History of heart attack[ia]	-0.036	0.121	-0.063	0.052	0.058	0.039	0.027	-0.287
History of stroke[ia]	-0.008	0.075	0.046	0.145	-0.039	-0.109	-0.123	-0.165
History of diabetes[ia]	0.012	0.197	-0.037	0.188	0.051	-0.178	0.044	-0.094
History of smoking[ia]	-0.024	-0.099	0.027	-0.055	0.016	0.193	-0.029	0.047
Elevated homocysteine level[ia]	0.087	0.042	-0.018	0.007	0.083	-0.102	-0.039	0.074
Total cholesterol[b]	0.011	-0.068	-0.097	-0.112	0.088	0.138	0.042	-0.024
Triglycerides[b]	0.004	0.040	**-0.148* (1.8%[l])**	0.013	0.070	-0.031	0.100	0.000
High density lipoprotein[b]	-0.011	-0.039	-0.014	-0.120	0.075	0.073	0.024	0.035
Low density lipoprotein[b]	0.015	-0.068	-0.060	-0.071	0.043	0.124	0.004	-0.041
High density lipoprotein large[c]	0.007	0.044	0.010	-0.013	0.055	-0.039	0.026	-0.063
High density lipoprotein intermediate[c]	-0.044	-0.153	-0.051	-0.159	0.095	0.157	0.038	0.062
High density lipoprotein small[c]	0.013	-0.014	-0.067	-0.188	0.070	0.160	0.008	0.244
Very low density lipoprotein[c]	0.020	-0.095	-0.075	-0.051	0.017	-0.065	0.113	-0.084
Intermediate density lipoprotein A[c]	0.015	-0.002	0.012	0.003	0.050	0.039	-0.022	-0.072
Intermediate density lipoprotein B[c]	0.024	-0.039	-0.056	-0.073	0.084	0.146	0.039	0.071
Intermediate density lipoprotein C[c]	0.047	-0.126	-0.097	-0.107	0.114	0.069	0.035	0.053
Low density lipoproteins 1 and 2[c]	0.030	-0.095	-0.057	-0.075	0.048	0.085	-0.015	0.025
Low density lipoproteins 3 – 7[c]	0.052	-0.019	-0.053	0.067	0.065	-0.023	0.080	0.041
Change in total cholesterol[b]	0.002	-0.080	0.029	0.021	-0.011	-0.080	0.003	-0.061
Change in triglycerides[b]	-0.065	-0.027	-0.081	-0.025	0.131	-0.008	0.019	-0.020
Change in high density lipoprotein[b]	0.054	-0.045	0.016	-0.019	-0.105	0.125	-0.006	-0.054
Change in low density lipoprotein[b]	-0.002	-0.069	0.035	0.039	0.001	-0.126	0.009	-0.050

[d]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. For the components of the CVD index analysed separately, the eight other components of the index are also included as covariates. Bold indicates statistical significance (**p* < 0.01). [i]Components of the CVD index analysed separately. [l]Portion of variance explained by diet score. [a]CVD index and individual components do not include body mass index as a covariate. [b]Measured by PathWest clinical pathology tests. [c]Baseline lipid subfractions determined using the Lipoprint system. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.7 *Odds ratios of the CVD index changing over 36 months for the cohort as a whole and following stratification of the cohort by gender and APOE ε4 allele carriage.*

Group[+]	Diet Pattern	Odds Ratio[a]	Confidence Interval[a]	*p*-value[a]	Odds Ratio[b]	Confidence Interval[b]	*p*-value[b]
Whole cohort	MeDi	0.64	0.331 – 1.247	0.191	0.70	0.349 – 1.412	0.321
Males	MeDi	0.63	0.228 – 1.740	0.372	0.87	0.284 – 2.670	0.808
Females	MeDi	0.54	0.216 – 1.375	0.198	0.62	0.244 – 1.591	0.323
***APOE* ε4 allele carrier**	MeDi	1.08	0.279 – 4.180	0.912	1.10	0.268 – 4.546	0.891
***APOE* ε4 allele non – carrier**	MeDi	0.57	0.261 – 1.236	0.154	0.62	0.274 – 1.406	0.253
Whole cohort	Prudent	1.01	0.527 – 1.929	0.981	2.05	1.050 – 4.000	0.035
Males	Prudent	0.70	0.266 – 1.840	0.469	1.25	0.457 – 3.398	0.667
Females	Prudent	1.25	0.486 – 3.213	0.643	3.66	1.368 – 9.776	**0.010***
***APOE* ε4 allele carrier**	Prudent	1.77	0.438 – 7.108	0.424	7.03	1.598 – 30.910	**0.010***
***APOE* ε4 allele non – carrier**	Prudent	0.85	0.399 – 1.795	0.664	1.57	0.720 – 3.434	0.256
Whole cohort	Western	0.81	0.399 – 1.645	0.559	0.52	0.245 – 1.090	0.083
Males	Western	2.04	0.676 – 6.181	0.205	0.80	0.227 – 2.822	0.730
Females	Western	0.44	0.154 – 1.273	0.131	0.34	0.120 – 0.972	0.044
***APOE* ε4 allele carrier**	Western	0.74	0.128 – 4.310	0.740	0.38	0.068 – 2.116	0.269
***APOE* ε4 allele non – carrier**	Western	0.87	0.393 – 1.930	0.734	0.55	0.231 – 1.296	0.171
Whole cohort	Inflammatory dietary index	1.37	0.637 – 2.947	0.420	0.60	0.279 – 1.297	0.195
Males	Inflammatory dietary index	1.63	0.534 – 4.973	0.391	1.06	0.321 – 2.779	0.926
Females	Inflammatory dietary index	0.57	0.199 – 1.616	0.288	0.35	0.117 – 1.055	0.062
***APOE* ε4 allele carrier**	Inflammatory dietary index	0.29	0.058 – 1.499	0.141	0.25	0.050 – 1.216	0.085
***APOE* ε4 allele non – carrier**	Inflammatory dietary index	1.17	0.508 – 2.677	0.716	0.83	0.339 – 2.038	0.687

Model includes age, gender, *APOE* ε4 allele carriage, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. [+]When the cohort is stratified by gender and *APOE* ε4 allele status, these characteristics are not included in the models as covariates. [a]Odds ratio, confidence interval and *p*-value for the odds of a decrease in the CVD index compared to no change in the CVD index over 36 months. [b]Odds ratio, confidence interval and *p*-value for the odds of an increase in the CVD index compared to no change in the CVD index over 36 months. Bold indicates statistical significance (***** $p < 0.01$). Multinomial logistic regression analysis used to calculate odds ratios. Abbreviations: *APOE*, Apolipoprotein E; CVD, cardiovascular disease; kCal, kilocalorie; MeDi, Mediterranean diet.

4.2.4. Diet scores and inflammation

4.2.4.1. Blood-based inflammation biomarker index 1

Inflammation was the third mechanism investigated as a possible explanation of the cognition and dietary pattern results observed in Chapter 3. A range of inflammatory biomarkers (cortisol, CRP, TNFα, complement component 3, cluster of differentiation 40 (CD40), CD40 ligand, migration inhibitory factor (MIF), macrophage inflammatory protein (MIP) 1 alpha, MIP 1 beta, TNF receptor II, immunoglobulin M (IgM), interleukin (IL) 3, IL4, IL8, IL10, IL13, IL18, interleukin 1 receptor antagonist) were analysed by Rules Based Medicine (RBM) at baseline using the Human DiscoveryMap® v1.0 panel (Austin, TX, USA). We constructed a blood-based inflammation biomarker index for each participant using these RBM biomarkers. There are no definitive published 'normal range' levels for most of these biomarkers, we consequently assigned a value of '1' to participants with biomarker levels falling in the top 20% of each biomarker measured (indicating a more inflammatory profile), participants where levels of each biomarker fell outside the top 20% were assigned a value of '0'. We then summed the values for each biomarker to construct a blood-based inflammation biomarker index with a score ranging from 0 to 18, with higher scores indicating a higher inflammatory profile. Linear regression analyses were conducted to assess the relationship of the four dietary patterns (independent variable) with the index, and additionally with the components of the index individually (dependent variables; Table 4.8). Consistent with the metabolic syndrome and CVD analyses reported in sections 4.2.2. and 4.2.3., the most common confounding variables were controlled for in all analyses, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, and energy intake.

The inflammatory dietary index was positively associated with the blood-based inflammation biomarker index in the cohort as a whole, i.e. as individuals consume less anti-inflammatory dietary components (indicated by a higher inflammatory dietary index), their blood-based inflammation biomarker index increases; the inflammatory dietary index explained 4.7% of the variance in the relationship ($\beta = 0.168$, $p = 0.008$; Table 4.8). Following stratification of the cohort by gender, this positive association was observed in

females only (β = 0.237, p = 0.007; Figure 4.1) with the inflammatory dietary index explaining 7.0% of the variance in the relationship. When the cohort was stratified by *APOE* ε4 allele carriage, a relationship approaching significance was observed in *APOE* ε4 allele non-carriers only (β = 0.166, p = 0.025; data not shown). We observed no associations between the blood-based inflammation biomarker index and the other three dietary patterns.

When evaluating the relationship between the four dietary patterns and the components of the index individually, we observed an unexpected positive association between MeDi adherence and IgM level (IgM is an antibody produced first in an immune response; β = 0.115, p = 0.009), with diet explaining 3.8% of the variance in this relationship. There were no associations with any of the dietary patterns and the other 17 biomarkers of inflammation in the cohort as a whole (Table 4.8). We once again repeated the analysis following stratification of the cohort by gender and *APOE* ε4 allele carriage; only one statistically significant relationship was observed, with the inflammatory dietary index positively associated with IL18 in males; the dietary index explained 7.1% of the variance in this relationship (β = 0.221, p < 0.010; data not shown).

Table 4.8: *Relationship between baseline diet scores and blood-based inflammation biomarker index 1: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score	Baseline prudent diet score	Baseline western diet score	Baseline inflammatory dietary index
Inflammation index	-0.045	-0.039	-0.018	**0.168* (4.7%[l])**
Cortisol[sa]	-0.015	-0.019	0.017	0.038
CRP[sb]	-0.018	-0.069	0.052	0.119
TNFα[sc]	0.005	0.001	-0.049	0.111
C3[sd]	-0.069	-0.098	0.044	0.092
CD40[se]	0.066	-0.012	0.017	-0.008
CD40 ligand[sf]	-0.062	-0.053	0.042	0.073
MIF[sg]	-0.068	-0.028	-0.025	0.040
MIP 1α[sh]	-0.058	-0.011	-0.056	0.073
MIP 1β[si]	-0.001	0.005	0.043	0.083
TNF RII[sj]	0.042	-0.016	0.025	0.003
IgM[sk]	**0.115* (3.8%[l])**	0.067	-0.027	-0.080
IL10[sl]	-0.021	0.020	-0.016	0.029
IL13[sm]	-0.044	0.024	-0.042	0.008
IL18[sn]	-0.011	-0.049	-0.018	0.095
IL1ra[so]	-0.074	-0.043	0.023	0.127
IL3[sp]	-0.017	0.016	-0.037	0.015
IL4[sq]	-0.048	0.032	-0.073	0.009
IL8[sr]	0.014	0.091	-0.109	0.019

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$). [l]Portion of variance explained by diet score. [a]A corticosteroid hormone produced by the adrenal gland. [b]A protein found in the blood in response to inflammation. [c]A cytokine involved in systemic inflammation and stimulating the acute phase reaction. [d]A protein that works with the immune system and plays a role in the development of inflammation. [e]A costimulatory protein found on antigen presenting cells, and is required for their activation. [f]A protein that is primarily expressed on activated T cells. [g]A lymphokine involved in cell mediated immunity, immunoregulation and inflammation. [h]A chemokine with pro-inflammatory activities, also inhibits the proliferation of hematopoietic stem cells. [i]A factor produced by macrophages that causes local inflammatory responses. [j]Receptors that bind TNFα. [k]Includes the antibodies that are usually produced first in an immune response which are later replaced by other types of antibodies. [l]Secreted by antigen-presenting cells, promotes the development of immunologic tolerance and suppresses the production of inflammatory cytokines. [m]A cytokine secreted by many cell types, but especially T helper type 2 cells, an important mediator of allergic inflammation and disease. [n]A cytokine that works together with IL12 to induce cell-mediated immunity following infection with microbial products. [o]An agent which binds to the same receptor on the cell surface as IL1R and prevents IL1 from sending a signal to that cell. [p]A cytokine that can improve the body's natural response to disease as part of the immune system. [q]A cytokine that induces differentiation of naïve helper T cells to TH2 cells. [r]A chemokine produced by macrophages and other cell types, an important mediator of the immune reaction in the innate immune system. [s]Components of the blood-based inflammation biomarker index 1 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; C3, complement component 3; CD40, cluster of differentiation 40; CRP, C-reactive protein; IgM, immunoglobulin M; IL, interleukin; IL1ra, interleukin 1 receptor antagonist; kCal, kilocalorie; MeDi, Mediterranean diet; MIF, migration inhibitory factor; MIP1α, macrophage inflammatory protein 1 alpha; MIP1β, macrophage inflammatory protein 1 beta; TNFRII, tumour necrosis factor receptor II; TNFα, tumour necrosis factor alpha.

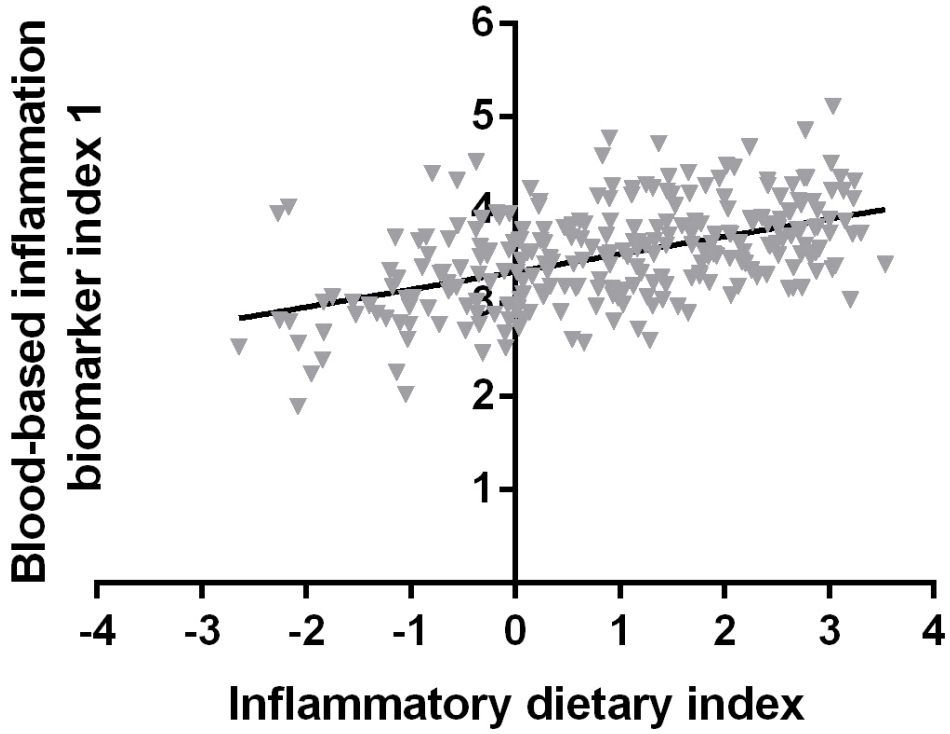

Figure 4.1: *Higher baseline inflammatory dietary index score is associated with higher blood-based inflammation biomarker index 1 score in females.*

Model adjusted for age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal (residuals plotted). Linear regression analysis. R^2_{adj} = 0.249, β = 0.237, *p* = 0.007, portion of variance = 7.0%. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie.

4.2.4.2. Blood-based inflammation biomarker index 2

4.2.4.2.1. Blood-based inflammation biomarker index 2 – cross-sectional analysis

We constructed a second blood-based inflammation biomarker index, based on ten analytes measured as part of the clinical tests conducted by PathWest laboratories at the baseline time-point and 36 month follow up (haemoglobin, red blood cell count, erythrocyte sediment rate, platelet count, white cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils). The index was computed using the same method as that employed to construct the blood-based inflammation biomarker index 1, i.e. we once again assigned a value of '1' to participants with biomarker levels falling in the top 20% of each biomarker measured, and a value of '0' to participants whose levels fell outside the top 20%; the scores for each biomarker were then summed to construct the blood-based inflammation biomarker index 2 with a value ranging from 0 to 10. Linear regression analyses were again used to assess the association of this blood-based inflammation biomarker index 2 and its individual components with the four dietary patterns in the cohort as a whole and following stratification of the cohort by gender and *APOE* ε4 allele carrier status.

The blood-based inflammation biomarker index 2 was positively correlated with western diet score in females ($\beta = 0.192$, $p = 0.002$; with diet explaining 3.9% of the variance in this relationship; Table 4.10 and Figure 4.2), and negatively correlated with prudent diet score in the whole cohort ($\beta = -0.136$, $p = 0.004$; with diet explaining 2.4% of the variance in this relationship; Table 4.9) as well as in females only ($\beta = -0.164$, $p = 0.010$; with diet explaining 2.9% of the variance in this relationship; Table 4.10 and Figure 4.2). Furthermore, the inflammatory dietary index was positively correlated with the blood-based inflammation biomarker index 2 in the cohort as a whole ($\beta = 0.181$, $p < 0.001$; with diet explaining 2.9% of the variance in this relationship; Table 4.9), as well as in *APOE* ε4 allele non-carriers ($\beta = 0.191$, $p = 0.004$; with diet explaining 3.9% of the variance in this relationship; Table 4.11 and Figure 4.3).

When evaluating the relationship between the four dietary patterns and the components of the index individually, several associations were observed (Tables 4.9 – 4.11). Briefly, the MeDi was 1) negatively correlated with platelet count in the cohort as a whole as well as in

APOE ε4 allele carriers; and 2) negatively associated with red blood cell count in females. Prudent diet was 1) negatively associated with white cell count in the cohort as a whole as well as in *APOE* ε4 allele non-carriers; 2) negatively associated with eosinophils in *APOE* ε4 allele carriers; 3) negatively associated with platelet count in females; and 4) positively associated with haemoglobin level in the cohort as a whole. Western diet was 1) positively associated with platelet count in the cohort as a whole as well as in females; 2) positively associated with eosinophils in *APOE* ε4 allele carriers; 3) positively associated with lymphocytes in females; and 4) negatively associated with haemoglobin and red blood cell count in the cohort as a whole as well as in both *APOE* ε4 allele non-carriers and in males. The inflammatory dietary index was 1) positively associated with white cell count in the cohort as a whole as well as in both males and *APOE* ε4 allele non-carriers; 2) positively associated with eosinophils in the whole cohort; 3) positively associated with neutrophil count in males; and 5) positively associated with lymphocyte level in females and *APOE* ε4 allele non-carriers.

Table 4.9: *Relationship between baseline diet scores and blood-based inflammation biomarker index 2: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score	Baseline prudent diet score	Baseline western diet score	Baseline inflammatory dietary index
Inflammation index 2	-0.003	**-0.136* (2.4%[†])**	0.090	**0.181** (2.9%[†])**
Haemoglobin[i]	-0.045	**0.108** (0.8%[†])**	**-0.179** (1.8%[†])**	-0.071
Red blood cell count[i]	-0.035	0.050	**-0.173** (1.8%[†])**	-0.014
Erythrocyte sediment rate[i]	0.039	-0.006	0.024	-0.012
Platelet count[i]	**-0.117* (2.1%[†])**	-0.116	**0.138* (2.0%[†])**	0.087
White cell count[i]	-0.026	**-0.135* (2.3%[†])**	0.122	**0.185** (3.0%[†])**
Neutrophils[i]	-0.033	-0.110	0.097	0.142
Lymphocytes[i]	0.008	-0.095	0.100	0.134
Monocytes[i]	-0.008	-0.065	0.053	0.099
Eosinophils[i]	-0.032	-0.075	0.031	**0.152* (2.5%[†])**
Basophils[i]	0.038	-0.071	0.058	0.077

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$, **$p < 0.001$). [†]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.10: *Relationship between baseline diet scores and blood-based inflammation biomarker index 2 following stratification of the cohort by gender: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
Biomarker	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Inflammation index 2	0.080	-0.043	-0.097	**-0.164* (2.9%[†])**	-0.010	**0.192* (3.9%[†])**	0.135	0.199
Haemoglobin[i]	0.082	-0.122	0.158	0.099	**-0.238* (9.4%[†])**	-0.143	-0.112	-0.040
Red blood cell count[i]	0.068	**-0.144* (2.4%[†])**	0.051	0.046	**-0.208* (7.0%[†])**	-0.128	-0.022	0.020
Erythrocyte sediment rate[i]	0.018	0.088	-0.059	0.037	0.030	0.007	-0.052	-0.021
Platelet count[i]	-0.091	-0.129	-0.071	**-0.170* (6.5%[†])**	0.068	**0.193* (6.7%[†])**	0.090	0.109
White cell count[i]	0.058	-0.073	-0.161	-0.114	0.083	0.145	**0.220* (6.5%[†])**	0.164
Neutrophils[i]	0.026	-0.077	-0.175	-0.054	0.104	0.072	**0.224* (7.7%[†])**	0.084
Lymphocytes[i]	0.090	-0.017	-0.021	-0.152	0.005	**0.166* (2.8%[†])**	0.053	**0.204* (2.8%[†])**
Monocytes[i]	0.072	-0.047	-0.066	-0.064	0.006	0.107	0.081	0.101
Eosinophils[i]	-0.005	-0.030	-0.026	-0.113	-0.052	0.137	0.100	0.163
Basophils[i]	0.022	0.030	-0.036	-0.123	0.066	0.073	0.108	0.078

Model includes age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$). [†]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.11: *Relationship between baseline diet scores and blood-based inflammation biomarker index 2 following stratification of the cohort by APOE ε4 allele carriage: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
***APOE* ε4 allele carrier status**[a]	-	+	-	+	-	+	-	+
Inflammation index 2	-0.014	0.047	-0.113	-0.173	0.061	0.146	**0.191*** **(3.9%[ł])**	0.094
Haemoglobin[i]	-0.008	-0.046	0.116	0.055	**-0.174**** **(1.7%[ł])**	-0.166	-0.062	-0.069
Red blood cell count[i]	-0.034	-0.049	0.071	-0.041	**-0.159*** **(1.6%[ł])**	-0.180	-0.017	0.028
Erythrocyte sediment rate[i]	0.057	0.023	-0.021	0.112	0.016	0.019	0.040	-0.265
Platelet count[i]	-0.069	**-0.220*** **(7.9%[ł])**	-0.088	-0.145	0.124	0.141	0.063	0.089
White cell count[i]	-0.045	0.010	**-0.147*** **(2.8%[ł])**	-0.086	0.120	0.107	**0.208*** **(4.0%[ł])**	0.057
Neutrophils[i]	-0.052	-0.008	-0.144	-0.092	0.084	0.126	0.153	0.065
Lymphocytes[i]	0.004	0.048	-0.135	0.066	0.143	-0.086	**0.182*** **(3.4%[ł])**	-0.078
Monocytes[i]	-0.001	-0.035	-0.041	-0.131	0.040	0.087	0.072	0.165
Eosinophils[i]	-0.053	0.036	-0.025	**-0.254*** **(6.5%[ł])**	-0.040	**0.312*** **(6.7%[ł])**	0.129	0.210
Basophils[i]	0.003	0.133	-0.100	-0.011	-0.088	-0.008	0.133	-0.071

[a]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$, **$p < 0.001$). [ł]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

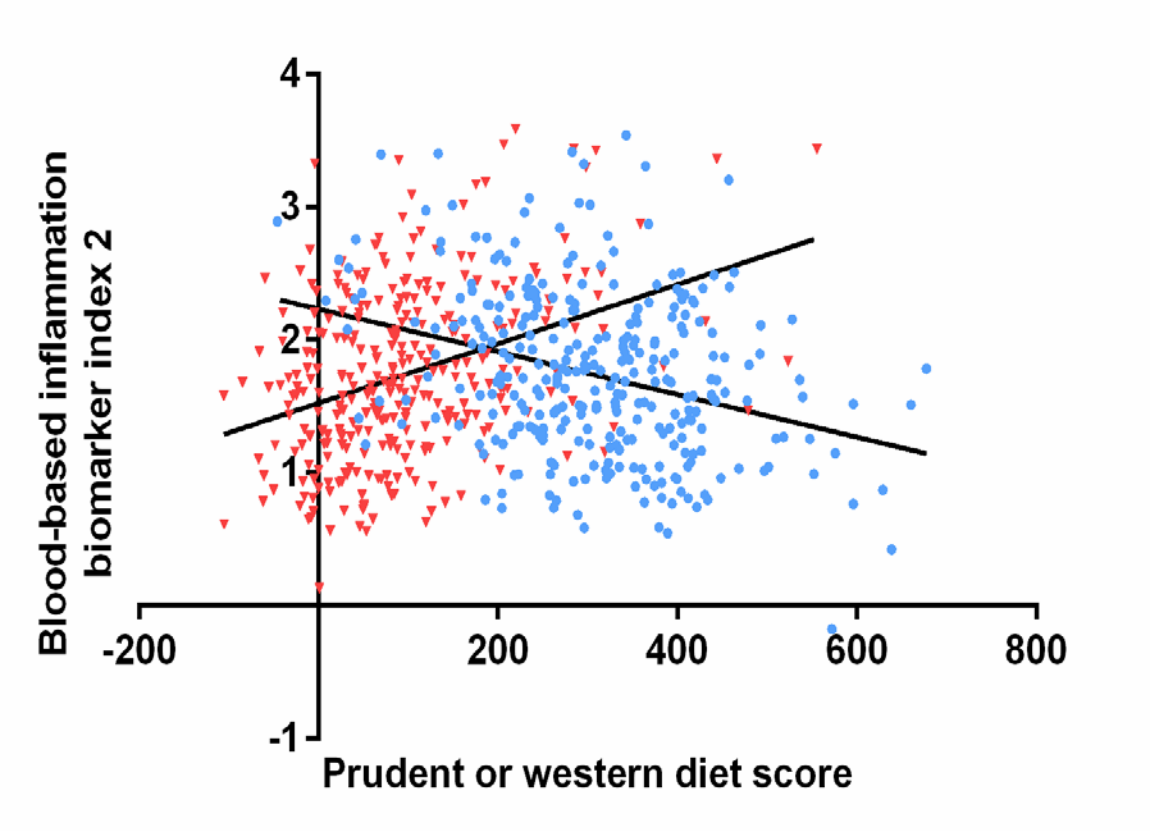

Figure 4.2: *Higher baseline western diet score (red triangles) and lower baseline prudent diet score (blue circles) is associated with higher blood-based inflammation biomarker index 2 level in females.*

Model adjusted for age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal (residuals plotted). Linear regression analysis: R^2_{adj} = 0.093, β = -0.164, *p* = 0.010, portion of variance = .9% for the prudent diet association, and R^2_{adj} = 0.127, β = 0.192, *p* = 0.002, portion of variance = 3.9% for the western diet association. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie.

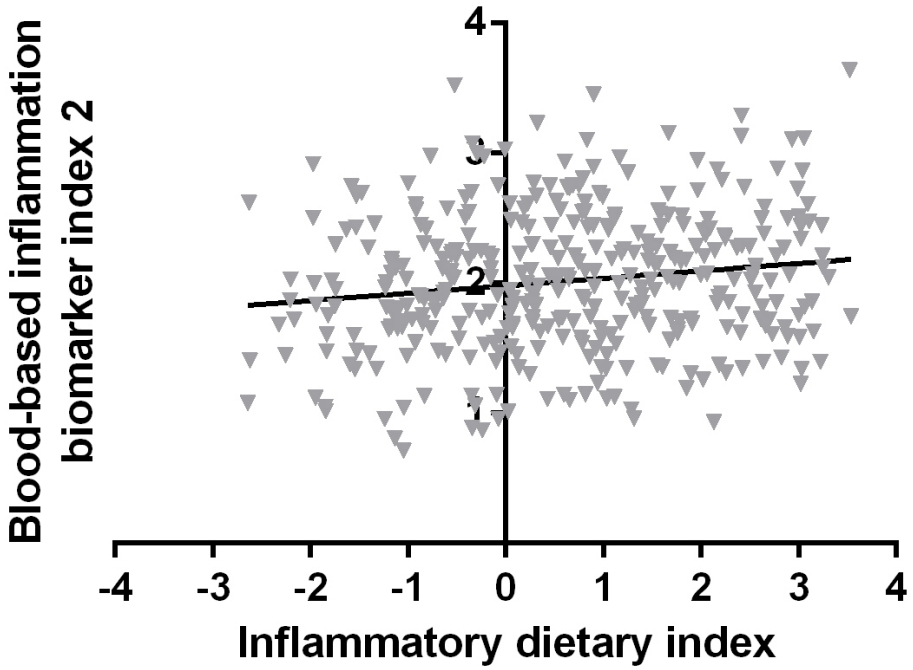

Figure 4.3: *Higher baseline inflammatory dietary index is associated with higher blood-based inflammation biomarker index 2 level in APOE ε4 allele non-carriers.*

Model adjusted for age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal (residuals plotted). Linear regression analysis: R^2_{adj} = 0.024, β = 0.191, *p* = 0.004, portion of variance = 3.9%. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie.

4.2.4.2.2. Blood-based inflammation biomarker index 2 – longitudinal analysis, change in index over 36 months

In order to assess the relationship of the four diet scores with inflammation longitudinally, a blood-based inflammation biomarker index 2 was also constructed for 36 month follow up data, using the same method as that utilised during construction of the baseline blood-based inflammation biomarker index 2. As per the longitudinal metabolic syndrome and CVD index analysis, the 36 month follow up inflammation index was subtracted from the baseline inflammation index score for each participant to give a value for 'change in the index' over 36 months. If the index increased over 36 months participants were assigned a value of '1', if there was no change participants were assigned a value of '2', and if the index decreased over 36 months, participants were assigned a value of '3'. This 'change in index' variable was then used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary adherence and change in inflammation level over 36 months ('no change' i.e. a score of '2', was the reference). The four diet scores were divided into tertiles for this analysis, as described in section 4.2.2.; odds ratios compared the lowest tertile with the highest tertile. The model was adjusted for the same potential confounders as those incorporated into the linear regression models described earlier. Although none of the odds ratios reached the significance level of $p < 0.01$, there was a trend towards greater likelihood of the blood-based inflammation biomarker index 2 increasing over 36 months if inflammatory dietary index score was in the highest tertile compared to the lowest adherence tertile in *APOE* ε4 allele non-carriers (odds ratio (OR) = 0.41; CI: 0.176 – 0.963; $p = 0.041$). There was also a trend towards greater likelihood of the blood-based inflammation biomarker index 2 increasing over 36 months if western diet adherence was in the highest tertile compared to the lowest adherence tertile in females (OR = 0.39; CI: 0.156 – 0.973; $p = 0.044$; Table 4.12).

Table 4.12: *Odds ratios of the blood-based inflammation biomarker index2 changing over 36 months for the cohort as a whole and following stratification of the cohort by gender and APOE ε4 allele carriage.*

Group[+]	Diet Pattern	Odds Ratio[a]	Confidence Interval[a]	*p*-value[a]	Odds Ratio[b]	Confidence Interval[b]	*p*-value[b]
Whole cohort	MeDi	1.03	0.575 – 1.855	0.913	1.55	0.852 - 2.842	0.151
Males	MeDi	1.03	0.419 – 2.550	0.942	1.31	0.489 – 3.523	0.590
Females	MeDi	1.00	0.445 – 2.233	0.995	1.81	0.824 – 3.977	0.139
***APOE* ε4 allele carrier**	MeDi	1.4	0.418 – 4.689	0.586	1.46	0.491 – 4.363	0.495
***APOE* ε4 allele non – carrier**	MeDi	0.92	0.465 – 1.819	0.810	1.69	0.800 – 3.559	0.169
Whole cohort	Prudent	1.23	0.698 – 2.176	0.471	1.71	0.922 – 3.175	0.089
Males	Prudent	1.29	0.541 – 3.059	0.568	1.53	0.583 – 4.016	0.388
Females	Prudent	1.13	0.512 – 2.506	0.758	1.77	0.760 – 4.126	0.816
***APOE* ε4 allele carrier**	Prudent	2.22	0.650 – 7.546	0.203	2.75	0.776 – 9.768	0.117
***APOE* ε4 allele non – carrier**	Prudent	1.06	0.547 – 2.032	0.873	1.42	0.686 – 2.915	0.347
Whole cohort	Western	0.85	0.460 – 1.580	0.612	0.62	0.323 – 1.206	0.160
Males	Western	1.27	0.481 – 3.357	0.629	1.26	0.426 – 3.715	0.679
Females	Western	0.70	0.287 – 1.715	0.438	0.39	0.156 – 0.973	0.044
***APOE* ε4 allele carrier**	Western	0.66	0.158 – 2.727	0.562	0.30	0.075 – 1.242	0.097
***APOE* ε4 allele non – carrier**	Western	0.96	0.474 – 1.929	0.902	0.92	0.408 – 1.881	0.794
Whole cohort	Inflammatory dietary index	1.19	0.611 – 2.320	0.608	0.53	0.260 – 1.065	0.074
Males	Inflammatory dietary index	0.88	0.318 – 2.455	0.813	0.54	0.176 – 1.625	0.270
Females	Inflammatory dietary index	1.85	0.714 – 4.719	0.207	0.55	0.207 – 1.470	0.234
***APOE* ε4 allele carrier**	Inflammatory dietary index	0.80	0.186 – 3.448	0.766	1.16	0.285 – 4.696	0.839
***APOE* ε4 allele non – carrier**	Inflammatory dietary index	1.44	0.666 – 3.134	0.352	0.41	0.176 – 0.963	0.041

Model includes age, gender, *APOE* ε4 allele carriage, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. [+]When the cohort is stratified by gender and *APOE* ε4 allele status, these characteristics are not included in the models as covariates. [a]Odds ratio, confidence interval and *p*-value for the odds of a decrease in the inflammation index compared to no change in the inflammation index over 36 months. [b]Odds ratio, confidence interval and *p*-value for the odds of an increase in the inflammation index compared to no change in the inflammation index over 36 months. Multinomial logistic regression analysis used to calculate odds ratios. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

4.2.4.2.3. Blood-based inflammation biomarker index 2 – cross-sectional analysis, baseline diet scores associated with 36 month follow-up index

The cross-sectional results reported in section 4.2.4.2.1. suggest a strong relationship between diet and blood-based biomarkers of inflammation. The longitudinal results reported in section 4.2.4.2.2. however, report no change in blood-based inflammation biomarker index 2 over 36 months in association with high or low dietary pattern adherence. It is conceivable that no change in index over 36 months would be observed in relation to diet pattern adherence if levels of inflammatory markers remained consistently high or low. To further investigate this hypothesis, we used linear regression analysis to assess the relationship between baseline diet scores, the 36 month follow up blood-based inflammation biomarker index 2 and its individual components.

We did not observe any associations between the 36 month follow up blood-based inflammation biomarker index 2 and the baseline dietary patterns in the cohort as a whole or following stratification of the cohort by gender and *APOE* ε4 allele carriage. There were however, several associations seen in relation to individual components of the 36 month follow up index and the baseline dietary patterns which were also observed with respect to the baseline index. Briefly, these consistent results include; 1) inflammatory dietary index positively associated with white cell count in the cohort as a whole and in *APOE* ε4 allele non-carriers; 2) prudent diet adherence positively associated with haemoglobin level in the cohort as a whole; 3) western diet negatively associated with haemoglobin level in the cohort as a whole; 4) western diet adherence negatively associated with red cell count in the cohort as a whole; 5) western diet positively associated with platelet level in the cohort as a whole; 6) western diet adherence positively associated with lymphocyte level in females; and 7) prudent diet negatively associated with eosinophil level in *APOE* ε4 allele carriers. We also observed additional associations at 36 months, including; 1) inflammatory dietary index positively associated with neutrophil count in the cohort as a whole; 2) prudent diet score association with haemoglobin level in the whole cohort was also seen in males and *APOE* ε4 allele non-carriers; 3) prudent diet negatively associated with neutrophil count in males; 4) the association between western diet adherence and lymphocyte level in females

was also seen in *APOE* ε4 allele non-carriers; and 5) western diet adherence was positively associated with basophil level in females. Tables 4.13 to 4.15 show the regression results in the cohort as a whole, and following stratification of the cohort by gender and *APOE* ε4 allele carriage respectively.

Table 4.13: *Relationship between baseline diet scores and 36 month follow-up blood-based inflammation biomarker index 2: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score	Baseline prudent diet score	Baseline western diet score	Baseline inflammatory dietary index
Inflammation index 2	0.015	-0.066	0.060	0.107
Haemoglobin[i]	-0.020	**0.114* (1.1%[ł])**	**-0.141* (1.3%[ł])**	-0.097
Red blood cell count[i]	-0.016	0.085	**-0.136* (1.1%[ł])**	-0.038
Erythrocyte sediment rate[i]	0.023	-0.012	0.018	-0.039
Platelet count[i]	-0.068	-0.079	**0.143* (1.3%[ł])**	0.018
White cell count[i]	0.000	-0.113	0.093	**0.179* (1.7%[ł])**
Neutrophils[i]	-0.020	-0.075	0.035	**0.158* (1.3%[ł])**
Lymphocytes[i]	0.033	-0.100	0.136	0.084
Monocytes[i]	0.017	-0.042	0.048	0.079
Eosinophils[i]	0.009	-0.090	0.044	0.135
Basophils[i]	-0.020	-0.056	0.078	0.026

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$). [ł]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.14: *Relationship between baseline diet scores and 36 month follow-up blood-based inflammation biomarker index 2 following stratification of the cohort by gender: linear regression analysis. Standardised β values shown.*

	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
Biomarker	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Inflammation index 2	0.093	-0.001	-0.044	-0.078	-0.034	0.153	0.136	0.046
Haemoglobin[i]	0.047	-0.089	**0.204* (3.4%[ł])**	0.077	-0.205	-0.111	-0.205	-0.018
Red blood cell count[i]	0.073	-0.103	0.134	0.051	-0.161	-0.123	-0.104	0.046
Erythrocyte sediment rate[i]	0.021	0.042	-0.024	0.009	-0.033	0.055	-0.028	-0.063
Platelet count[i]	-0.033	-0.081	-0.048	-0.101	0.099	0.165	0.026	0.009
White cell count[i]	0.132	-0.063	-0.197	-0.057	0.154	0.019	0.198	0.172
Neutrophils[i]	0.100	-0.091	**-0.226* (4.2%[ł])**	0.031	0.167	-0.099	0.195	0.141
Lymphocytes[i]	0.089	0.032	-0.016	-0.154	0.057	**0.182* (2.5%[ł])**	0.052	0.109
Monocytes[i]	0.071	-0.027	-0.004	-0.084	-0.007	0.100	0.045	0.127
Eosinophils[i]	0.079	-0.028	-0.065	-0.127	-0.025	0.168	0.142	0.090
Basophils[i]	0.030	-0.061	-0.019	-0.141	0.032	**0.178* (2.3%[ł])**	0.068	0.021

Model includes age, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$). [ł]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

Table 4.15: *Relationship between baseline diet scores and 36 month follow-up blood-based inflammation biomarker index 2 following stratification of the cohort by APOE ε4 allele carriage: linear regression analysis. Standardised β values shown.*

Biomarker	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
***APOE* ε4 allele carrier status**[a]	-	+	-	+	-	+	-	+
Inflammation index 2	0.093	-0.001	-0.044	-0.078	-0.034	0.153	0.136	0.046
Haemoglobin[i]	0.047	-0.089	**0.204* (1.4%[ł])**	0.077	-0.205	-0.111	-0.205	-0.018
Red blood cell count[i]	0.073	-0.103	0.134	0.051	-0.161	-0.123	-0.104	0.046
Erythrocyte sediment rate[i]	0.021	0.042	-0.024	0.009	-0.033	0.055	-0.028	-0.063
Platelet count[i]	-0.033	-0.081	-0.048	-0.101	0.099	0.165	0.026	0.009
White cell count[i]	0.132	-0.063	-0.197	-0.057	0.154	0.019	0.198	0.172
Neutrophils[i]	0.100	-0.091	**-0.226* (1.7%[ł])**	0.031	0.167	-0.099	0.195	0.141
Lymphocytes[i]	0.089	0.032	-0.016	-0.154	0.057	**0.182* (2.4%[ł])**	0.052	0.109
Monocytes[i]	0.071	-0.027	-0.004	-0.084	-0.007	0.100	0.045	0.127
Eosinophils[i]	0.079	-0.028	-0.065	-0.127	-0.025	0.168	0.142	0.090
Basophils[i]	0.030	-0.061	-0.019	-0.141	0.032	**0.178* (1.3%[ł])**	0.068	0.021

[a]*APOE* ε4 allele carriage - is *APOE* ε4 allele non-carrier, + is *APOE* ε4 allele carrier. Model includes age, gender, years of education, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Bold indicates statistical significance (*$p < 0.01$). [ł]Portion of variance explained by diet score. [i]Components of the blood-based inflammation biomarker index 2 analysed separately. Abbreviations: *APOE*, Apolipoprotein E; kCal, kilocalorie; MeDi, Mediterranean diet.

4.2.5. Diet scores and neuroimaging biomarkers

In AD, specific areas of the brain show signs of atrophy when examined using structural MRI. In general, there is widespread cortical atrophy, yet those areas involved with processing primary motor and sensory information are spared. Those areas most likely to show atrophy are parts of the medial and lateral temporal lobes, and the frontal lobes (338-343). In PiB PET studies of MCI and AD individuals, higher PiB retention in these people (indicative of cerebral Aβ load) is associated with greater cognitive decline (43, 51, 344, 345). Additionally, approximately 30% of 'healthy' individuals over the age of 75 years show Aβ deposition at autopsy (346, 347). This supports the belief that neuropathological changes precede the clinical expression of AD by many years (348), and it is hypothesised that this group represents preclinical AD (349). Studies show that 20 – 25% of cognitively 'normal' elderly participants demonstrate higher cortical PiB retention (48, 49, 350), and collectively, these studies suggest that early intervention trials for AD should target these individuals who demonstrate significant cerebral Aβ deposition (49).

The influence of the *APOE* ε4 allele in increasing AD risk is reported by such studies, for example, in a group of 177 healthy older adults, 33% had high PiB retention; those with high PiB retention were on average 5 years older and twice as likely to carry an *APOE* ε4 allele (55). It has also been found that carriers of the *APOE* ε4 allele experience accelerated brain atrophy compared with non-carriers (351, 352). Considering this evidence collectively, together with the *APOE* genotype-dependent effects of diet on cognition in cognitively healthy individuals (some of whom would be 'classified' as preclinical AD) reported in Chapter 3, we investigated the relationship between dietary pattern adherence and neuroimaging measures of brain volume (MRI) and cerebral Aβ load (PiB PET): the influence of *APOE* genotype on these relationships was also assessed.

4.2.5.1. Demographics of the neuroimaging cohort

As mentioned in section 4.2.1., a subgroup of the healthy control cohort utilised for the cognition (Chapter 3) and blood biomarker analysis (Chapter 4) underwent brain imaging: Table 4.16 lists the demographics of this subgroup. The average age of the neuroimaging cohort at baseline was 70.6 ± 7.2 and nearly 50% were males. Almost 40% of these individuals carried at least one *APOE* ε4 allele, over 45% had 12 or less years of education and 78.9% were born in Australia. Mean BMI was 26.3 ± 4.0 and mean energy intake was 1706.9 ± 568.6 kCal. In relation to the neuroimaging measures, the mean hippocampal, grey matter, white matter, ventricle and cerebrospinal fluid (CSF) volumes were 3.1 cubic centimetres (cm^3), 668.7cm^3, 436.3cm^3, 29.6cm^3 and 411.2cm^3 respectively. The mean PiB PET standardised uptake value ratio (SUVR) was 1.4 ± 0.4. The average baseline MeDi score of the subgroup was 4.2 ± 1.5, the average prudent diet score was 299.7 ± 126.5, the average western diet score was 146.6 ± 143.8, and the average inflammatory dietary index was 0.6 ± 1.5. There were several differences observed between males and females. Females had a higher average inflammatory dietary index ($p < 0.001$; ANOVA). As expected, males had a higher energy intake ($p < 0.001$; ANOVA). Males also had a higher western diet score ($p < 0.001$; ANOVA). When the healthy control neuroimaging subgroup was stratified by *APOE* ε4 allele carriage, *APOE* ε4 allele carriers were shown to have a higher western diet score ($p < 0.001$; ANOVA) and a higher mean PiB PET SUVR ($p < 0.001$; ANOVA; Table 4.16). Figure 4.4 shows the distribution of baseline (blue line), 18 month follow up (black line) and 36 month follow up PiB PET SUVR (red line) for each of the four dietary patterns constructed at baseline. Figure 4.5 shows the distribution of PiB PET SUVR at baseline (blue box plot), 18 month (black box plot) and 36 month follow up (red box plot) for each baseline dietary pattern divided into tertiles.

Table 4.16: *Descriptive statistics for the neuroimaging healthy control cohort as a whole, and subgroups following stratification of the cohort by gender and APOE ε4 allele carriage.*

	Whole cohort (n = 123)	Males (n = 60)	Females (n = 63)	*p*-values for gender differences	*APOE* ε4 carrier (n = 49)	*APOE* ε4 non-carrier (n = 74)	*p*-values for *APOE* ε4 carriage differences
Age at baseline; y	70.6 ± 7.2	70.6 ± 7.2	70.7 ± 7.2	0.910	68.9 ± 6.9	71.8 ± 7.2	0.029
Gender, men; n (%)	60 (48.8)				28 (57.1)	32 (43.2)	0.131
Country of birth, Australian; n (%)	97 (78.9)	47 (78.3)	50 (79.4)	0.889	39 (79.6)	58 (78.4)	0.872
Presence of *APOE* ε4 allele; n (%)	49 (39.8)	28 (46.7)	21 (33.3)	0.131			
Baseline body mass index[i]; kg/m²	26.3 ± 4.0	26.5 ± 4.3	26.2 ± 3.7	0.687	26.2 ± 3.9	26.4 ± 4.1	0.819
Baseline energy intake; kCal	1706.9 ± 568.6	1972.3 ± 596.9	1454.0 ± 404.9	**0.000**	1802.1 ± 591.3	1643.8 ± 548.0	0.131
Education, ≤ 12 y; n (%)	57 (46.3)	26 (43.3)	31 (49.2)	0.514	29 (59.2)	28 (37.8)	0.020
Baseline MRI hippocampal volume; cm³ (n = 111)	3.1 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	0.055	3.1 ± 0.2	3.2 ± 0.2	0.186
Baseline MRI grey matter volume; cm³ (n = 111)	668.7 ± 23.1	666.2 ± 29.3	670.9 ± 15.7	0.290	669.5 ± 21.1	668.2 ± 24.4	0.772
Baseline MRI white matter volume; cm³ (n = 111)	436.3 ± 36.5	439.3 ± 38.8	433.6 ± 34.5	0.415	445.6 ± 34.4	430.4 ± 36.8	0.032
Baseline MRI ventricle volume; cm³ (n = 111)	29.6 ± 14.2	28.4 ± 16.3	30.6 ± 12.2	0.416	29.3 ± 16.5	29.8 ± 12.7	0.873
Baseline MRI CSF volume; cm³ (n = 111)	411.2 ± 39.7	413.4 ± 41.2	409.4 ± 38.6	0.599	401.5 ± 35.8	417.4 ± 41.1	0.039
Baseline PiB PET SUVR (n = 123)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.819	1.5 ± 0.5	1.3 ± 0.3	**0.000**
Baseline MeDi score	4.2 ± 1.5	4.4 ± 1.6	4.0 ± 1.4	0.162	4.4 ± 1.5	4.1 ± 1.5	0.217
Baseline prudent diet score	299.7 ± 126.5	311.1 ± 135.6	288.8 ± 117.3	0.330	282.3 ± 128.9	311.2 ± 124.5	0.215
Baseline western diet score	146.6 ± 143.8	213.4 ± 156.0	82.9 ± 95.4	**0.000**	197.2 ± 159.2	113.1 ± 122.7	**0.001**
Baseline inflammatory dietary index	0.6 ± 1.5	0.2 ± 1.4	1.1 ± 1.4	**0.001**	0.6 ± 1.4	0.7 ± 1.5	0.975

Unless otherwise described, data are presented as mean ± standard deviation of the mean. Bold indicates statistical significance ($p < 0.01$); Characteristics compared using analysis of variance for continuous variables and χ^2 for categorical variables. [i]Body mass index is calculated as weight in kilograms divided by height in meters squared. Abbreviations: *APOE*, Apolipoprotein E; cm^3, cubic centimetres; CSF, cerebrospinal fluid; kCal, kilocalorie; Kg, kilogram; m^2, meter squared; MeDi, Mediterranean diet; MRI, magnetic resonance imaging; PiB PET, Pittsburgh compound-B positron emission tomography; SUVR, standardised uptake value ratio; y, years.

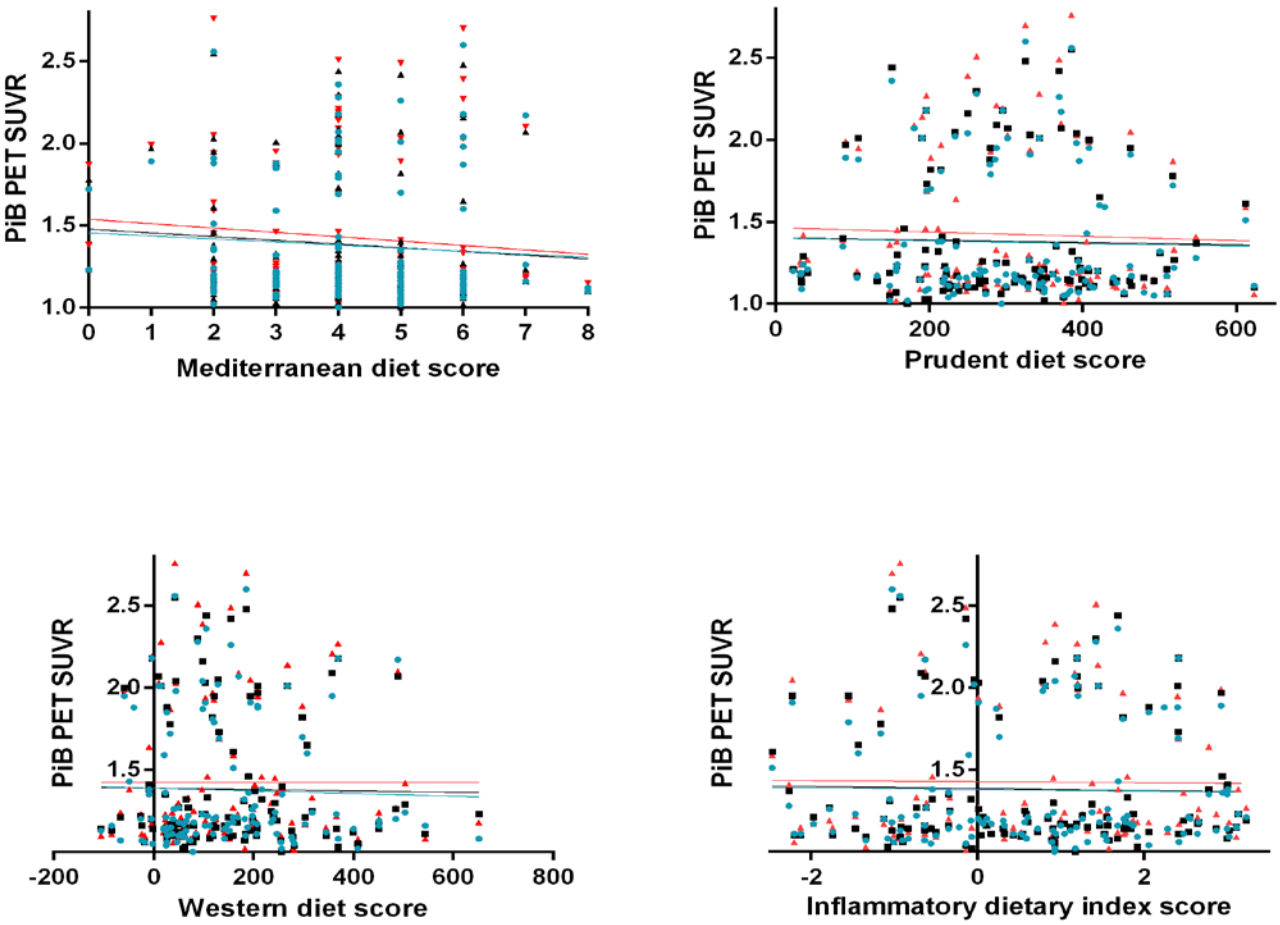

Figure 4.4: *Distribution of baseline PiB PET SUVR (blue symbols and line), 18 month follow up PiB PET SUVR (black symbols and line) and 36 month follow up PiB PET SUVR (red symbols and line) for each of the four dietary patterns constructed at baseline.*

Linear regression analysis; $p > 0.05$. Abbreviations: PiB PET, Pittsburgh Compound-B positron emission tomography; SUVR, standardised uptake value ratio.

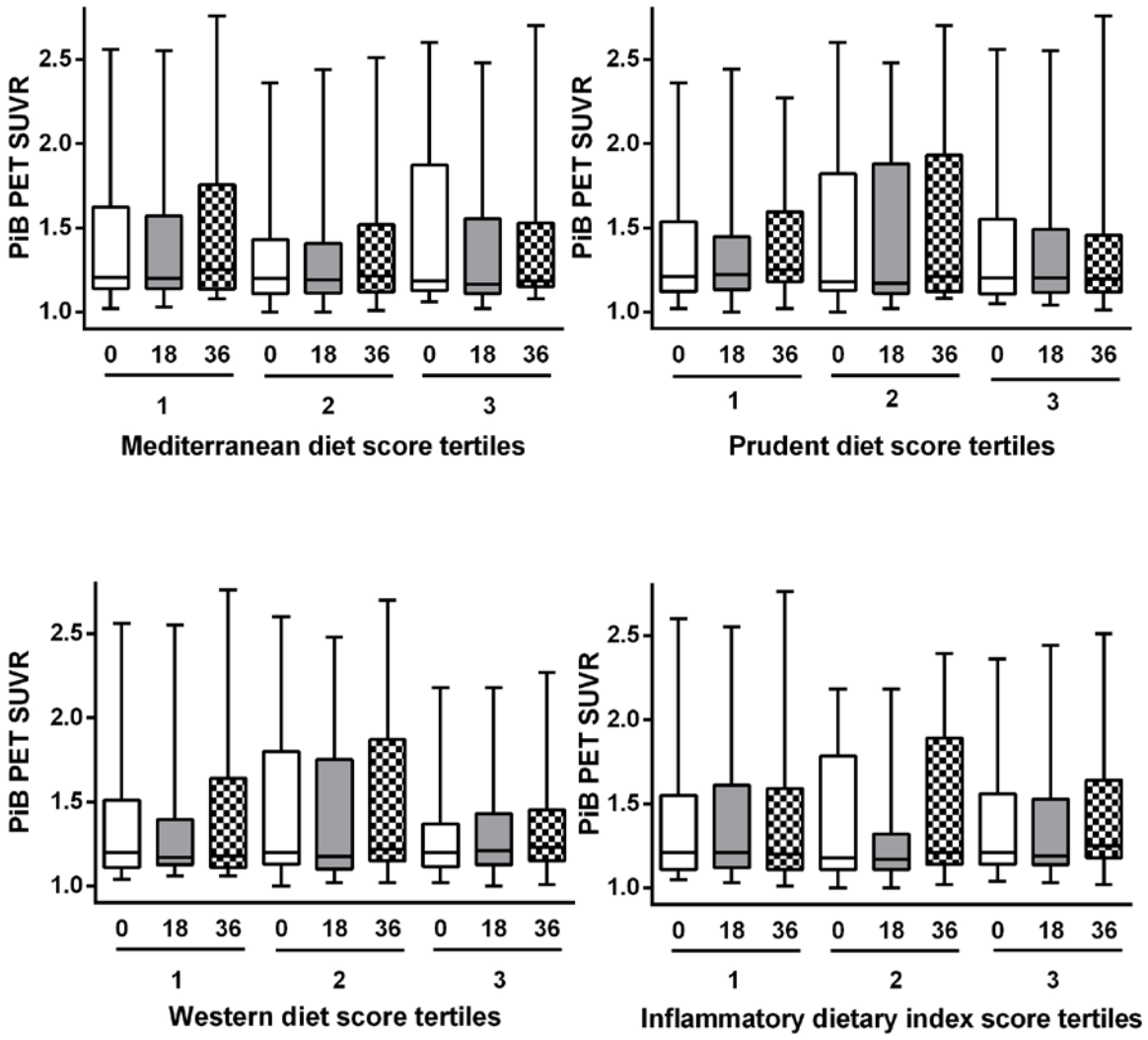

Figure 4.5: *Distribution of PiB PET SUVR at baseline (0; blue box plot), 18 month follow up (18; black and white box plot) and 36 month follow up (36; red box plot) for each baseline dietary pattern divided into tertiles. Tertile 1 = lowest adherence; Tertile 3 = highest adherence.*

Independent samples t-tests*; p* > 0.05. Abbreviations: PiB PET, Pittsburgh Compound-B positron emission tomography; SUVR, standardised uptake value ratio.

4.2.5.2. Dietary patterns, brain volumes and cerebral amyloid load

Multiple linear regression analyses were run to determine the association between the four continuous diet scores and MRI-determined brain region volumes. After controlling for the most common confounding variables, i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, BMI, and energy intake, there were no significant associations observed between baseline diet scores and baseline MRI volumes (data not shown). We were particularly interested in assessing whether baseline diet scores could predict change in regional brain volumes. Consequently, we calculated a change score for each MRI brain region, by subtracting the baseline value from the 36 month follow up value for each individual. These 'change' scores were then used in multiple linear regression models to investigate their association with the baseline diet scores. No associations were found between these 'change' variables and baseline diet scores in the cohort as a whole, or when stratified by gender or APOE ε4 allele carriage (data not shown).

We further investigated these associations following stratification of the cohort by amyloid status, i.e. PiB PET negative (SUVR < 1.5) or PiB PET positive (SUVR ≥ 1.5), as it can be argued that the PiB PET positive individuals within the healthy control group represent a biologically distinct subgroup within whom the processes associated with AD pathogenesis are more advanced: However, no significant associations were observed following this stratification (Table 4.17). There were associations trending towards significance, including western diet approaching a negative association with ventricle volume in PiB PET positive individuals (β = -0.559, p = 0.047), and MeDi adherence approaching a negative association with change in hippocampal volume (β = -0.696, p = 0.015), also in PiB PET positive individuals. It is possible that small sample size limited statistical power when investigating these relationships. Figure 4.6 shows the mean diet scores at baseline for each of the four dietary patterns following stratification of the cohort into PiB PET positive and PiB PET negative groups.

We also assessed the relationship between PiB PET determined cerebral Aβ load (SUVR) and the baseline dietary patterns whilst again controlling for the most common confounding variables listed above. There were no significant associations between PiB PET SUVR and

the diet scores at baseline. To assess the relationship between brain Aβ load and diet score longitudinally, we computed a change in PiB PET SUVR variable by subtracting the baseline SUVR from the 36 month follow up SUVR for each individual, however we found no association between this 'change in SUVR' variable and the baseline diet scores. Furthermore, following stratification of the cohort by gender, APOE ε4 allele carriage (data not shown) and PiB PET status i.e. PiB PET positive or negative (Table 4.17), no significant associations between cerebral Aβ load and diet scores were observed. There were some associations trending towards significance, for example, MeDi adherence approaching a negative association with change in PiB PET SUVR in *APOE* ε4 allele non-carriers (β = -0.273, p = 0.047; data not shown). When stratified by PiB PET status, the inflammatory dietary index was trending towards a positive association with change in SUVR in PiB PET negative individuals; i.e. those with lower consumption of anti-inflammatory foods at baseline (indicated by a higher inflammatory dietary index) have greater increase in amyloid load over the next 36 months (β = 0.329, p = 0.035; Table 4.17). The opposite was true for PiB PET positive individuals (β = -0.690, p = 0.035; Table 4.17), and we propose that as they already have high amounts of amyloid in their brain, dietary factors can no longer have an effect on SUVR. Prudent diet was also trending towards a negative association with change in PiB PET SUVR amongst PiB PET negative individuals (β = -0.297, p = 0.024); i.e. as prudent diet adherence increases, there is less accumulation of cerebral amyloid over 36 months (Table 4.17).

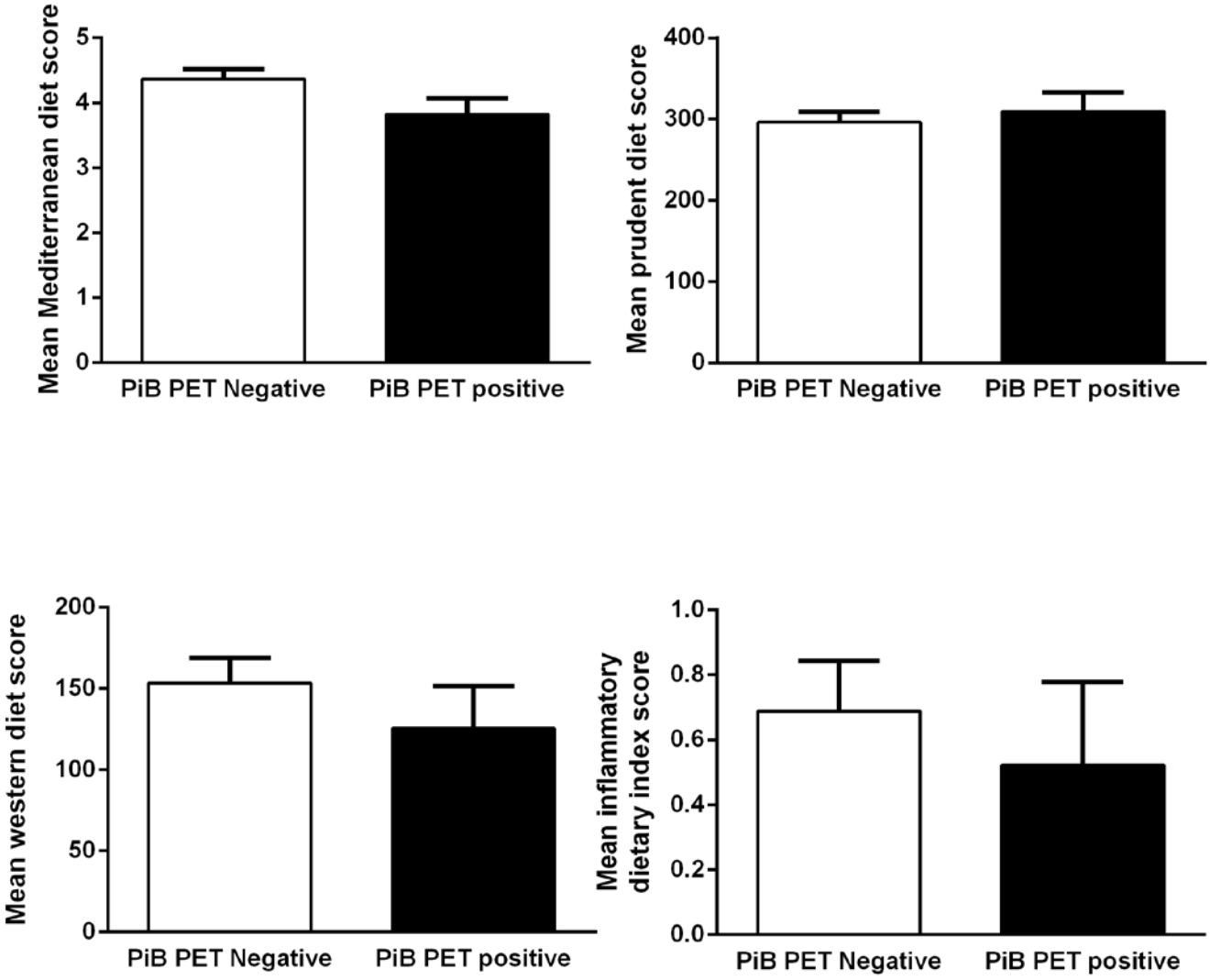

Figure 4.6: *Mean diet scores at baseline for each of the four dietary patterns following stratification of the cohort into PiB PET positive and PiB PET negative groups.*

Data represented as mean ± SEM. Independent samples t-tests*; p* > 0.05. PiB PET negative – SUVR < 1.5; PiB PET positive – SUVR ≥ 1.5. Abbreviations: PiB PET, Pittsburgh Compound-B positron emission tomography; SEM, standard error of the mean; SUVR, Standardised uptake value ratio.

Table 4.17: *Relationship between baseline dietary patterns and baseline MRI volumes, change in MRI volumes over 36 months, baseline PiB PET SUVR and change in PiB PET SUVR over 36 months, following stratification of the cohort by PiB PET status: linear regression analysis. Standardised β values shown.*

PiB PET positive or negative[a]	-	+	-	+	-	+	-	+
MRI brain region	Baseline MeDi score		Baseline prudent diet score		Baseline western diet score		Baseline inflammatory dietary index	
MRI hippocampus[b]	-0.138	-0.265	-0.021	-0.022	-0.064	0.041	0.045	0.037
MRI grey matter[b]	-0.086	-0.303	0.106	-0.289	-0.056	0.230	0.145	0.103
MRI white matter[b]	0.064	0.194	-0.027	0.064	-0.054	-0.052	1.000	0.330
MRI ventricle[b]	0.140	-0.226	-0.065	0.195	0.130	-0.559	-0.024	-0.056
MRI CSF[b]	-0.006	-0.071	-0.035	0.076	0.099	-0.081	-0.104	-0.335
Change in MRI hippocampus[b]	0.193	-0.696	-0.025	-0.371	-0.214	0.918	-0.187	0.049
Change in MRI grey matter[b]	0.081	-0.068	0.088	-0.224	-0.278	0.452	-0.161	-0.138
Change in MRI white matter[b]	-0.233	-0.179	-0.044	0.034	0.052	0.429	-0.074	-0.171
Change in MRI ventricle[b]	0.167	-0.342	-0.197	-0.201	0.119	0.379	0.168	-0.087
Change in MRI CSF[b]	0.135	0.365	-0.039	0.196	0.123	-0.705	0.235	0.248
PiB PET SUVR continuous score	-0.100	0.556	-0.083	-0.285	-0.007	0.244	0.130	0.208
Change in PiB PET SUVR continuous score	-0.200	0.188	-0.297	0.046	0.205	-0.043	0.329	-0.690

[a]PiB PET positive or negative, - is PiB PET negative (SUVR < 1.5), + is [a]PiB PET positive (SUVR ≥ 1.5). [b]MRI brain region volumes corrected for intracranial volume. Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), baseline body mass index and baseline energy intake kCal as covariates. Abbreviations: *APOE*, Apolipoprotein E; CSF, cerebrospinal fluid; kCal, kilocalorie; MeDi, Mediterranean diet; MRI, magnetic resonance imaging; PiB PET, Pittsburgh compound-B positron emission tomography; SUVR, standardised uptake value ratio.

Finally, we assigned a value of '0' to participants classified as PiB PET negative (SUVR < 1.5) and a value of '1' to participants classified as PiB PET positive (SUVR ≥ 1.5). This binary score (with PiB PET negative as the reference) was used in multinomial logistic regression models to calculate odds ratios for the investigation of the association between baseline dietary pattern adherence and classification as either PiB PET negative or positive. The four diet scores were again divided into tertiles for this analysis; odds ratios compared the lowest tertile with the highest tertile. For the MeDi score, individuals with a score of 3 or lower (lowest adherence to MeDi) were assigned to tertile 1, those with a score of 4 or 5 were assigned to tertile 2, and those with a score of 6 or higher (highest adherence to MeDi) were assigned to tertile 3. For the inflammatory dietary index, western and prudent diets, the participants' scores were arranged into increasing order of adherence and then divided equally into thirds, with the lowest adherence third to each pattern assigned to tertile 1, the middle third assigned to tertile 2 and the highest adherence third assigned to tertile 3. The model was adjusted for the same potential confounders as those incorporated into the linear regression models described earlier in this section. Dietary pattern adherence did not affect the likelihood of being PiB PET positive when comparing the highest tertile to the lowest adherence tertile for each of the four dietary patterns, in the cohort as a whole or following stratification of the cohort by gender and *APOE* ε4 allele carriage (data not shown).

4.3. Discussion

In this chapter, we sought to explore potential mechanisms that might underlie the effects of diet on cognition reported in Chapter 3 by assessing the relationship of the dietary patterns (MeDi, western diet, prudent diet, and inflammatory dietary index) with blood and neuroimaging biomarker data collected as part of the AIBL study. A plethora of potential mechanisms exist, we focussed our investigations on factors that are well-established contributors to AD risk i.e. metabolic syndrome, CVD, and inflammation. We also assessed the relationship between the dietary patterns and MRI-determined brain volume and PiB PET-determined cerebral amyloid load.

4.3.1. Dietary patterns and metabolic syndrome

Metabolic syndrome is a disorder of energy utilization and storage, diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal (central) obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low HDL levels. To investigate the association of the four dietary patterns with metabolic syndrome risk, we utilised the parameters listed above to construct a metabolic syndrome index. We also assessed the relationship of the dietary patterns with the components of the index individually as well as with additional metabolic syndrome biomarker data collected as part of the AIBL study.

The main findings reported in this section were;

There were no associations between the four baseline dietary patterns and the metabolic syndrome index, or the individual components of the index in the cohort as a whole or following stratification of the cohort by gender or *APOE* ε4 allele carriage.

Longitudinal analysis revealed that the likelihood of the metabolic syndrome index increasing or decreasing over 36 months was unaffected by dietary pattern adherence, in the cohort as a whole and following stratification of the cohort by gender and *APOE* ε4 allele carriage.

Investigation of the cross-sectional relationship of the dietary patterns with additional biomarkers of metabolic syndrome (measured only at baseline) revealed that insulin levels were negatively associated with prudent diet score in the cohort as a whole. Glucagon was negatively associated with western diet score in males and *APOE* ε4 allele non-carriers. Further, glucagon-like peptide-1 was negatively associated with western diet score also in *APOE* ε4 allele non-carriers.

Glucagon-like peptide-1 is an incretin hormone which regulates postprandial glucose levels through glucose-dependent insulin secretion: It is expressed in neurons and acts as a neurotransmitter. Additionally, glucagon-like peptide-1 has been shown to demonstrate growth factor-like properties, to protect neurons from neurotoxic influences, to reduce the induction of apoptosis of hippocampal neurons, and improve spatial and associated learning (353). It is perhaps unsurprising therefore, that adherence to the 'unhealthy' western diet was associated with decreased levels of this beneficial hormone.

Metabolic syndrome and diabetes are inextricably linked. Type 1 diabetes is characterised by a deficit in the production of insulin, and has been shown to increase cognitive dysfunction in a review of 33 longitudinal studies (354). Type 2 diabetes is caused by resistance to the effects of insulin, and is often fuelled by obesity which is believed to be caused by impaired response to leptin. Insulin and leptin exert their effects through complex signalling cascades and both pathways share some common components (355). Leptin and insulin both have roles in the regulation and maintenance of physiological homeostasis, both hormones have been shown to regulate neuronal and synaptic functions within the hippocampus, cortex and cerebellum, both hormones protect neurons against neurodegeneration and cell death (356-359) and also regulate Aβ levels by modulating Aβ production and Aβ degradation (115, 360-362).

A number of clinical and epidemiological studies have provided evidence of a link between type 2 diabetes, obesity and AD (114, 117, 120, 363-373). Possible mechanisms to explain the link between type 2 diabetes and AD include; causing increased risk of metabolic syndrome (the majority of type 2 diabetes patients are obese and together with

type 2 diabetes this can lead to the development of hypertension, an additional feature of metabolic syndrome); causing hyperglycaemia which may have toxic effects on neurons, and can lead to oxidative stress; and by causing hyperinsulinemia which in itself is a risk factor for AD (105, 106, 374). A large body of literature implicates hyperinsulinemia and insulin resistance in AD risk (375), with one study reporting a doubled risk of AD in people with high insulin levels (110, 284). Amongst individuals with chronically high peripheral insulin levels, brain insulin levels are also high, and one reason this may increase AD risk is that degradation of brain Aβ is inhibited due to the competition with insulin for insulin-degrading enzyme (376).

Dietary modification has the potential to reduce the risk of progression of prediabetic conditions such as metabolic syndrome to type 2 diabetes (377, 378), primarily through weight loss (379). Recommendations to reduce weight are a mainstay of diabetes care and have been formally recommended as the foundation on which all additional diabetes therapies should rest. High calorie intake and diets high in sugar, added sweeteners and refined flour are major health concerns in the western world, and along with sedentary lifestyles have led to the growing incidence of obesity. Ajala *et al*. (380) reviewed studies of dietary patterns most suitable for reducing weight, improving glycaemic control and lipid profiles in people with type 2 diabetes. The authors concluded that low carbohydrate, low glycaemic index, Mediterranean and high protein diets are effective. High fibre, vegetarian and vegan diets were not found to be effective. Another meta-analysis by Liveley *et al*. (381) also found increasing dietary glycaemic load was associated with increased risk for type 2 diabetes, although others have advised that a low glycaemic load diet should not be a recommendation for decreasing type 2 diabetes risk due to imprecision of published glycaemic index data from which dietary glycaemic load is calculated (382). Individual studies and a systematic review show that adherence to a MeDi decreases glycated haemoglobin, fasting blood glucose and the need for antihyperglycemic drug therapy (230, 383-388). However, we report no relationship between MeDi adherence, metabolic syndrome index and additional biomarkers of metabolic syndrome. Conversely, consistent with the literature, we found cross-sectional associations between prudent diet

adherence and decreased insulin level, and western diet adherence and decreased glucagon (*APOE* ε4 allele non-carriers and males) and glucagon-like peptide-1 (*APOE* ε4 allele non-carriers).

There is evidence that control of hypertension (a component of our metabolic syndrome index) is associated with a reduced risk of cognitive impairment and dementia, which is likely to be due to a reduction in CVD, atherosclerosis and stroke (76, 105, 389, 390). Some, but not all, studies have suggested that good control of hypertension is associated with cognitive benefit and decreased AD neuropathology, although this is age-dependent (109, 391-401). Several clinical trials found no association with hypertension medication and cognitive performance (105, 402-404). Positive lifestyle modifications may contribute to management in prehypertensive and treated hypertensive patients (405). The benefits of diet on hypertension have been emphasised in several studies, in particular a protective effect of fruit, vegetables, legumes, whole grains, fish, nuts and low-fat dairy products (the MeDi is composed of many of these foods; 406, 407-418). Vegetarians have also been shown to have lower blood pressures than non-vegetarians (419). Another study with evidence of a beneficial effect of dietary intake on blood pressure is the Dietary Approaches to Stop Hypertension clinical trial (408). In this trial, participants were fed a control diet low in fruit, vegetables and dairy products, or a diet with a fat content typical of the average United States of America diet. Participants were then assigned to either; 1) the control diet; 2) a diet rich in fruit and vegetables; or 3) a combination diet rich in fruit, vegetables and low fat dairy products with reduced saturated and total fat. The combination diet lowered both systolic and diastolic blood pressure, and the fruit and vegetables diet lowered systolic blood pressure in comparison to the control diet. These results show a low fat diet, rich in fruit and vegetables, with adequate intake of calcium, magnesium and potassium is required to reduce blood pressure, and individual nutrients are unlikely to be effective on their own (408). However, in contrast to the studies described here, we found no association with any of the four dietary patterns and history of hypertension or elevated blood pressure.

Whilst we report associations between dietary pattern adherence and several of the additional biomarkers of metabolic syndrome measured at baseline, our lack of significant associations between the four dietary patterns and our metabolic syndrome index as well as the individual components of the index, suggests that modulation of metabolic syndrome risk may not be the primary mechanism underlying the effects of diet on cognition described in chapter 3 of this thesis.

4.3.2. Dietary patterns and cardiovascular disease

CVD refers to any disease or condition of the heart and blood vessels. The causes of CVD are diverse but atherosclerosis and/or hypertension are the most common. To investigate the association of the four dietary patterns with CVD risk, we constructed a CVD index based on the presence of each of the following significant CVD risk factors; obesity, raised blood pressure, a history of angina, stroke, heart attack, smoking and diabetes, low physical activity and elevated homocysteine level. We also assessed the relationship of the dietary patterns with the components of the index individually as well as with additional CVD biomarker data collected as part of the AIBL study.

The main findings reported in this section were;

The index and its individual components were not associated with the four dietary patterns in the cohort as a whole or following stratification of the cohort by gender or *APOE* ε4 allele carriage.

Longitudinal analysis revealed that prudent diet adherence affected the likelihood of the CVD index remaining stable over 36 months. Being in the highest tertile of prudent diet adherence gave females and *APOE* ε4 allele carriers higher odds of their CVD index remaining at the baseline level rather than increasing at 36 months compared to the lowest adherence tertile.

Investigation of the cross-sectional relationship of the dietary patterns with additional biomarkers of CVD risk revealed that IDL-C was positively associated with western diet

score in females. Further, higher adherence to prudent diet at baseline was associated with decreased triglyceride level in males and *APOE* ε4 allele non-carriers.

There were no associations between dietary pattern adherence and change in the levels of the additional biomarkers of CVD risk over 36 months in the cohort as a whole or following stratification of the cohort by gender or *APOE* ε4 allele carriage.

As discussed in Chapter 1, an increase in disorders associated with metabolic syndrome and CVD can increase an individual's probability of developing AD (115, 156, 284, 376, 420-423), and this consequently represents a potential mechanism by which diet can affect AD risk and progression. For example, high levels of circulating cholesterol, triglycerides and LDL and low levels of HDL have been associated with increased AD risk (76, 78, 424-428). There is much evidence to suggest that raising HDL levels reduces risk of CVD, AD, and certain cancers (428, 429). Interestingly, the MeDi has been shown to have beneficial effects on the cholesterol-HDL cholesterol ratio in a randomised trial of 772 individuals compared to a low fat diet (418), and specific components of the MeDi, including olive oil and nuts, have been shown to improve lipid profiles (430, 431). However, we report no relationship between MeDi adherence, CVD index and additional biomarkers of CVD. Interestingly it was adherence to the other 'healthy' dietary pattern, namely the prudent diet, which increased the likelihood of the CVD index remaining stable rather than increasing over 36 months in females and *APOE* ε4 allele carriers. The MeDi and prudent diet are both heavily loaded with fruit and vegetables, thus we can only hypothesise that it is their differences (e.g. incorporation of cereals, dairy and alcohol intake into MeDi construction) that account for the disparate results reported in relation to CVD index.

The results of Chapter 3 reveal beneficial effects of MeDi adherence and detrimental effects of western diet adherence on cognition over 36 months. Whilst we report associations between dietary pattern adherence and biomarkers of CVD in this chapter, our lack of significant associations between the MeDi and western diet patterns and our CVD index as well as the individual components of the index, again suggests that modulation of

CVD risk may not be the primary mechanism underlying the effects of diet on cognition described in chapter 3 of this thesis.

4.3.3. Dietary patterns and inflammation

Inflammation is heavily implicated in AD pathology. To investigate the association of the four dietary patterns with inflammation we constructed two blood-based indexes of inflammation. The first index included a range (n = 18) of inflammatory biomarkers (including interleukins and cytokines) which were analysed at baseline only by Rules Based Medicine using the Human DiscoveryMap® v1.0 panel (Austin, TX, USA). The second index was based on ten analytes measured as part of the clinical tests (including measurement of types of white blood cells etc.) conducted by PathWest laboratories at baseline and 36 month follow up. We also assessed the relationship of the dietary patterns with the components of the indexes individually.

The main findings reported in this section were;

The first index was positively associated with the inflammatory dietary index in females; suggesting that the foods we consume affect levels of blood-based inflammatory biomarkers. When analysing the components of the index individually, we saw an association between increased MeDi adherence and increased IgM level, which is surprising as 'healthy' MeDi adherence is proposed to be associated with lower inflammation levels. Following stratification of the cohort by gender, the inflammatory dietary index was positively associated with IL18 in males.

The second blood-based inflammation biomarker index was found to be positively associated with western diet score in females, negatively associated with prudent diet score in the cohort as a whole as well as in females, and positively associated with the inflammatory dietary index in the cohort as a whole as well as in *APOE* ε4 allele non-carriers. These results are consistent with consumption of the 'healthy' prudent diet being associated with reduced levels of inflammation and conversely, adherence to the 'unhealthy' western diet and low level consumption of anti-inflammatory foods (indicated

by a higher inflammatory dietary index) being associated with increased levels of blood-based biomarkers of inflammation.

The dietary patterns were correlated with many individual components of the second index of inflammation. Briefly; higher MeDi score was associated with lower levels of inflammatory biomarkers in the whole cohort (platelet count) and in females (red blood cell count). Higher prudent diet score was also associated with lower levels of inflammatory biomarkers (white cell count) in *APOE* ε4 allele non-carriers, in *APOE* ε4 allele carriers (eosinophil levels) and in females (platelet count). Higher western diet score was associated with increased inflammatory biomarker levels in females (platelet count and lymphocyte level), and in *APOE* ε4 allele carriers (eosinophil level). The inflammatory dietary index was positively associated with increased levels of a number of the inflammatory biomarkers, namely; white cell count in males and *APOE* ε4 allele non-carriers, eosinophil level in the whole cohort, neutrophil level in males and lymphocyte level in females and *APOE* ε4 allele non-carriers.

Longitudinal analysis revealed that the likelihood of the second blood-based inflammation biomarker index increasing or decreasing over 36 months was not significantly affected by dietary pattern adherence, in the cohort as a whole and following stratification of the cohort by gender and *APOE* ε4 allele carriage. Although none of the odds ratios reached the significance level of $p < 0.01$, there was a trend towards greater likelihood of the blood-based inflammation biomarker index 2 increasing over 36 months if inflammatory dietary index score was in the highest tertile compared to the lowest adherence tertile in *APOE* ε4 allele non-carriers ($p = 0.041$), and a trend towards greater likelihood of the second blood-based inflammation biomarker index increasing over 36 months if western diet adherence was in the highest tertile compared to the lowest adherence tertile in females ($p = 0.044$).

The cross-sectional results reported in this chapter suggest a strong relationship between diet and blood-based biomarkers of inflammation. The longitudinal however, report no change in blood-based inflammation biomarker index 2 over 36 months in association with high or low dietary pattern adherence. It is conceivable that no change in index over 36

months would be observed in relation to diet pattern adherence if levels of inflammatory markers remained consistently high or low. To further investigate this hypothesis, we used linear regression analysis to assess the relationship between baseline diet scores, the 36 month follow up blood-based inflammation biomarker index 2 and its individual components.

We did not observe any associations between the 36 month follow up blood-based inflammation biomarker index 2 and the baseline dietary patterns in the cohort as a whole or following stratification of the cohort by gender and *APOE* ε4 allele carriage. There were however, several associations seen in relation to individual components of the 36 month follow up index and the baseline dietary patterns which were also observed with respect to the baseline index. Briefly, these consistent results include; 1) inflammatory dietary index positively associated with white cell count in the cohort as a whole and in *APOE* ε4 allele non-carriers; 2) prudent diet adherence positively associated with haemoglobin level in the cohort as a whole; 3) western diet negatively associated with haemoglobin level in the cohort as a whole; 4) western diet adherence negatively associated with red cell count in the cohort as a whole; 5) western diet positively associated with platelet level in the cohort as a whole; 6) western diet adherence positively associated with lymphocyte level in females; and 7) prudent diet negatively associated with eosinophil level in *APOE* ε4 allele carriers. We also observed additional associations at 36 months, including; 1) inflammatory dietary index positively associated with neutrophil count in the cohort as a whole; 2) prudent diet negatively associated with neutrophil count in males; 3) the association between western diet adherence and lymphocyte level in females was also seen in *APOE* ε4 allele non-carriers; and 4) western diet adherence was positively associated with basophil level in females.

The associations between food, nutrients and some diseases (including AD) may be mediated in part through inflammation. As discussed in Chapter 1, the inflammation process caused by the aggregation of Aβ in AD involves the production of inflammatory molecules including cortisol, CRP and interleukins; this inflammation reflects the development and progression of disease. CRP has been used in many studies as a

biomarker of inflammation; it is an acute phase protein produced in the liver and its levels are up-regulated by pro-inflammatory cytokines like interleukin 6 (IL6; 432) and TNFα. In a study of 1524 men and 1518 women, those in the higher MeDi adherence tertile had on average a 20% lower CRP level ($p = 0.015$), a 17% lower IL6 level ($p = 0.025$), a 15% lower homocysteine level ($p = 0.031$) and a 14% lower white cell count ($p < 0.001$) compared with those in the lowest tertile. The association with TNFα did not reach significance (p = 0.076; 214). Gu *et al.* (227) also found higher adherence to the MeDi was associated with lower levels of CRP ($p < 0.003$) in 1219 non-demented elderly participants. Fung *et al.* (296) used an alternate MeDi (this alternate MeDi excludes dairy, has a nut group, has only red and processed meat in the meat section and excludes potatoes) and concluded that those in the top adherence quintile compared to the bottom quintile had a 24% lower CRP concentration. A protective effect of adherence to the MeDi was found by Panagiotakos *et al.* (297); for each unit of increasing adherence to the MeDi, there was a reduction of 3.1% in the average CRP level and 1.9% in the average IL6 level. An intervention study with 90 participants following a MeDi and 90 following a prudent diet found participants that consumed the MeDi compared to the prudent diet had significantly reduced serum concentrations of CRP, IL6, IL7 and IL18 as well as decreased insulin resistance (231). However, not all studies have found positive results for these inflammatory biomarkers, Dai *et al.* (331) found higher adherence to the MeDi was associated with reduced levels of IL6 but had no significant effect on CRP. In an intervention study, 42 healthy male university students had either an alcohol free MeDi or a high fat diet for 90 days; between days 30 and 60 both diets were supplemented with 240 ml/day red wine. Neither diet nor wine supplementation had a detectable effect on CRP levels (332), however the cohort was not a large one.

Few studies have examined the contribution of other major dietary patterns to markers of systemic inflammation. In a cross-sectional study of healthy Iranian women, after adjusting for potential confounders, a 'healthy' dietary pattern was inversely related to plasma concentrations of CRP ($p < 0.05$) and vascular cell adhesion molecule 1 ($p < 0.05$). The western diet score constructed by these researchers was positively related to serum amyloid

A ($p < 0.05$) and IL6 ($p < 0.001$; 300). Fung *et al*. (212) found significant positive correlations between their own western dietary pattern and insulin, CRP, leptin and homocysteine concentrations, and an inverse correlation with plasma folate concentrations. The prudent dietary pattern constructed by these authors was by contrast positively associated with plasma folate and inversely correlated with insulin and homocysteine concentrations. Nettleton *et al.* (299) derived four dietary patterns using factor analysis: The fats and processed meats pattern was positively associated with CRP (p for trend < 0.001), IL6 (p for trend < 0.001) and homocysteine (p for trend < 0.002). The beans, tomatoes and refined grains pattern was positively related to intercellular adhesion molecule 1 (ICAM1; p for trend < 0.007). The whole grains and fruit pattern was inversely associated with CRP, IL6, homocysteine (p for trend < 0.001) and ICAM1 (p for trend < 0.034), and the vegetables and fish pattern was inversely associated with IL6 (p for trend < 0.009). Collectively, these results suggest that a 'healthy' dietary pattern is related to lower levels of inflammatory markers.

Consistent with the reported literature, we found our 'unhealthy' western dietary pattern to be positively associated with levels of blood-based biomarkers of inflammation and our 'healthy' MeDi and prudent diet patterns to be negatively associated with levels of blood-based biomarkers of inflammation. We also found our inflammatory dietary index to be strongly positively correlated with the levels of numerous blood-based biomarkers of inflammation, i.e. as intake of anti-inflammatory foods decreased (indicated by a higher inflammatory dietary index), the levels of blood-based biomarkers of inflammation increased: a relationship that has never before been investigated in a well characterised ageing cohort. We observed many of these associations in both males and females as well as in *APOE* ε4 allele non-carriers and carriers.

As mentioned previously, the results of Chapter 3 reveal beneficial effects of MeDi adherence and detrimental effects of western diet adherence on cognition over 36 months. In this chapter we report strong associations between dietary pattern adherence and biomarkers of inflammation, and suggest that the interplay between diet and elevated chronic inflammation may contribute to the effects of diet on cognition described in

chapter 3 of this thesis. This hypothesis certainly warrants further investigation and it will become increasingly important to understand the 'pro-inflammatory' versus 'anti-inflammatory' contribution of each of the four dietary patterns investigated to further clarify whether inflammation is the primary candidate mechanism underlying the reported effects of diet on cognition.

4.3.4. Dietary patterns and neuroimaging

We investigated the relationship between dietary pattern adherence and neuroimaging biomarkers of AD, specifically; PiB PET-determined cerebral amyloid load and MRI-determined brain volume. There were no significant associations observed between the four dietary patterns, cerebral amyloid load, MRI brain region volumes, or change in these neuroimaging variables, when the cohort was analysed as a whole or following stratification by gender, *APOE* ε4 allele carriage or binary PiB PET status (i.e. significant amyloid load or reduced amyloid load). There were also no significant associations between the four dietary patterns divided into tertiles and PiB PET SUVR as a binary score (categorised as significant amyloid load or reduced amyloid load) when the cohort was analysed as a whole or following stratification of the cohort by gender and *APOE* ε4 allele carriage, although several associations were trending towards significance. It is important to note however, that only a subgroup of participants underwent neuroimaging thereby reducing the sample size from 527 to 123. This reduced sample size is likely to have limited the power to see associations of dietary pattern adherence with brain volumes and amyloid load, and a larger sample size may have seen the trending associations reach significance.

In clinical and research settings, MRI can be used to identify areas of the brain that have undergone changes associated with brain disease. Areas associated with memory including frontal and temporal lobes, and the hippocampus, all show signs of atrophy in AD patients (433). A study by Bobinski *et al.* (434) investigated the validity of using MRI in AD patients by studying the relationship between *post-mortem* hippocampal volume, determined by MRI and histological volumes. The authors found strong correlations

between MRI and histological measurements of the hippocampus, hippocampus/subiculum and hippocampus/parahippocampal gyrus (434). It is worthy of note however, that the participants utilised for analysis in this chapter are all categorised as 'cognitively healthy': A factor that may have also contributed to the unlikelihood of observing a significant relationship between brain volumes and dietary pattern adherence.

In the first human study to investigate the use of PiB in AD diagnosis, PiB retention (a measure of amyloid load) was two-fold greater in AD patients, when compared with cognitively healthy controls (51). In addition, a strong correlation between *post-mortem* histological measurement of brain Aβ plaque load and PiB binding has been reported (435). Higher PiB retention has also been associated with greater cognitive decline in older adults (349).

There are very few studies investigating the association between dietary patterns and PiB PET-determined cerebral amyloid load and MRI-determined brain region volumes. Titova *et al*. (335) investigated the individual components of the MeDi score in relation to grey matter, white matter, and the sum of grey and white matter (MeDi score was constructed at 70 years of age and MRI was undertaken at 75 years of age). A low consumption of meat and meat products was linked to a greater total brain volume (i.e. the sum of grey and white matter volume; $p < 0.03$). The MeDi score as a whole explained less variance in brain volumes than the single meat component, and the authors suggested that the MeDi score may mask possible associations of single MeDi components with brain health domains (335). Bowman *et al*. (436) constructed nutrient biomarker patterns (using plasma biomarkers of diet) and found a pattern high in plasma vitamins B, C, D and E was associated with higher total cerebral brain volume whilst a pattern high in trans-fat was associated with less total cerebral brain volume. Biomarkers linked to pathological processes which dietary factors affect have also been investigated, for example blood cholesterol levels have been linked to hippocampal and other brain grey matter volume (97, 333). Higher levels of homocysteine have been linked to greater white matter atrophy in older adults and individuals with mild cognitive impairment (437; vitamin B can be used to lower homocysteine levels and may slow the rate of brain atrophy, 438), and elevated

homocysteine has also been associated with hippocampal atrophy (439, 440). Obesity is associated with a below-average hippocampal size and high BMI in midlife is associated with an increased rate of hippocampal atrophy in late life (441-449). Furthermore, higher peripheral insulin has been associated with decreased brain atrophy (334).

Considered collectively, these results suggest that the relationship between brain volumes and dietary pattern adherence is worthy of further investigation in our cohort both longitudinally and with a larger sample size (facilitated by recent participant recruitment).

The paucity of information regarding cerebral amyloid load and diet also makes this relationship worthy of further investigation; an aim which can again be achieved in our cohort in the near future courtesy of collection of additional longitudinal data and supplementary cross-sectional data from new study participants.

4.3.5. Conclusions

The results presented in this chapter describe thorough characterisation of the relationship between the four dietary patterns (MeDi, prudent diet, western diet and inflammatory dietary index) and blood-based biomarkers of metabolic syndrome, CVD and inflammation as well as neuroimaging biomarkers of AD. A study of this nature has to our knowledge, not previously been undertaken.

Our results suggest that the interplay between diet and elevated chronic inflammation may contribute to the effects of diet on cognition described in chapter 3 of this thesis. Metabolic syndrome and CVD appeared to be less likely mechanistic candidates. The results of the neuroimaging analysis were limited by sample size. Additional work is required to further characterise these relationships.

We reported positive associations between MeDi and prudent diet adherence and blood-based biomarkers of inflammation and by contrast, negative associations between western diet and inflammatory dietary index and these biomarkers of inflammation. Whilst inflammation is a strong candidate mechanism, it is likely that a complex interplay exists with overlap of the effects of diet on several mechanisms concurrently. For example, the

strongest results in chapter 3 of this thesis were related to the beneficial effects of MeDi adherence on cognition. However, the MeDi did not appear to be highly correlated to any of the potential mechanisms investigated in this chapter. It may be that the MeDi exerts its protective effects via independent mechanisms not investigated here, or perhaps more likely is the possibility that rather than one pronounced mechanism of action, the MeDi acts via multiple mechanisms of (weaker level) action which synergistically confer protection. Both are plausible explanations that warrant further investigation. In addition to increasing sample size and utilising data from further longitudinal assessments, measurement of nutrient markers in the blood may help to further elucidate the potential mechanisms of action of diet.

Chapter Five

Validation of the modified Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire

5.1. Introduction

Food frequency questionnaires (FFQs) are a relatively inexpensive method of measuring usual food intake over an extended period, particularly among large cohorts. FFQs are easy to administer and have low subject burden and therefore are the most practical method for dietary data collection. Measuring diet has long been a challenge because many factors affect dietary intake recall, including age, gender, weight, ethnicity, period over which intake is recalled, and number and type of questions asked. FFQs are more practical for large-scale studies, compared with 24-hour recall methods and collection of food records which require trained interviewers. In Australia two FFQs are frequently used, the Cancer Council of Victoria food frequency questionnaire (CCVFFQ; used in Chapters 3 and 4 of this thesis; 450) and the Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire (CSIROFFQ; 451, 452).

Whilst the CCVFFQ utilised in Chapters 3 and 4 of this thesis assesses intake of 74 food and beverage items, the CSIROFFQ is a quantified FFQ containing over 200 items including single foods, mixed dishes, beverages and alcohol. We utilised the online version of the CSIROFFQ in this study, which has serving sizes that can be altered by the participants and a format capable of modification to allow inclusion of additional questions. The online CSIROFFQ includes a series of qualitative questions related to food preparation and cooking techniques, the use of salt, the use of sugar in beverages, the type of milk used, the type of bread consumed and the type of fats used as spread or in cooking. To avoid fatigue, the online FFQ was designed to have the foods arranged into categories and automatically saves data entered, allowing participants to leave the questionnaire and return later to complete it in multiple sittings. The online form eliminates incomplete questionnaires, as participants cannot move to the next section until all questions are fully answered in the previous section. The paper form of the CSIROFFQ has previously been validated (255-258, 453), but to our knowledge no validation has been completed for the online version. Participant responses to the online version are analysed by CSIRO using food composition derived from the Australian Food and Nutrient Database 2007 (AUSNUT 2007) and the British Food Composition Tables. AUSNUT 2007 contains analytical data published in Nutrient Tables for use in Australia 2006 (NUTTAB 2006) as well as data from the Food Standards Australia New Zealand

2006 (FSANZ 2006) analytical program, nutrient data from overseas food composition tables, food label information, imputed data from similar foods and data calculated using a recipe approach. The CSIROFFQ takes approximately 45 minutes to complete, requires access to the internet, and like the CCVFFQ, assesses usual daily intake over the preceding 12 months.

Accurate assessment of *overall* dietary intake is crucial to understanding the association between diet and chronic diseases: incorrect information may lead to false associations between dietary factors and diseases. There is a need to develop and validate methods of assessing food and beverage intake to facilitate the development of guidelines for the prevention of diet-related chronic disease. However, dietary questionnaires need to be both specific to the population being analysed, and to the disease of interest: currently, there is no Alzheimer's disease (AD)-specific FFQ available.

Accuracy in measuring intake of *individual* dietary constituents is also an important issue in the analysis and evaluation of results from studies of the association between diet and disease; because dietary questionnaires do not necessarily estimate true food intake, their validity needs to be determined. The most common method of validating a dietary questionnaire is through comparison of responses from the FFQ to actual food intakes documented for a limited number of days using weighed food records (259). It is also important to determine whether a FFQ provides reproducible results. 'Reproducibility' can also be thought of as 'reliability', and this is generally assessed by administering the FFQ at two time points to the same group of people and assessing the level of agreement between the two responses (454). We modified the online CSIROFFQ to include questions relating to intake of food items of interest to AD research (detailed information on the items included can be found in section 2.9.3.). Table 5.1 below summarises the questions relating to intake of food items of interest in AD research added to or altered in the original online CSIROFFQ to produce the modified CSIROFFQ utilised in this validation study.

Table 5.1: *Food items of interest in Alzheimer's disease research: questions relating to intake of these items were added to or altered in the original online CSIROFFQ to produce the modified CSIROFFQ utilised in the validation study.*

Food item
Chocolate type (milk, dark or white)
Increased number of oils added (grape seed, coconut etc.)
Wine type
Green and white tea
Pomegranates and pomegranate juice
Type of grapes (green or red/black)
Type of berries
Kidney, liver and heart
Type of meat used in stew/casserole/curry/goulash
Type of rice (white or brown)
Type of nuts and seeds
Herbs and spices consumed
Supplements consumed

Abbreviations: CSIROFFQ, Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire.

5.1.1. Methods

This study was undertaken by a subset of Perth-based AIBL participants (n = 49). In summary, the online modified CSIROFFQ was administered at two time-points, on average seven days apart. Participants completed a four-day weighed food record in between completion of the first and second administration of the questionnaire; recording commenced on a Sunday and finished on the following Wednesday. All participants were classified as cognitively 'healthy' controls at the time they completed the validation study.

5.1.2. Chapter aims

The aim of this chapter was to determine the suitability of the modified CSIROFFQ for use in studies of AD through; 1) assessment of 'reliability' of the modified CSIROFFQ by comparing the responses of individuals when the questionnaire was administered on two occasions, and 2) validation of the modified CSIROFFQ relative to four-day weighed food records. Following completion of the 'reliability' and validation assessments, we evaluated the relationship between intake of the added food items, cognitive data, blood-based biomarkers of inflammation and cerebral amyloid load.

We hypothesise that:

1. The modified CSIROFFQ is a reliable assessment tool, as demonstrated by the correlation of food and beverage intakes from two administrations of the modified questionnaire.

2. The modified CSIROFFQ is a valid tool for quantifying intake of foods of interest in AD research, as demonstrated by the correlation of the modified questionnaire responses to intake from the four-day weighed food records.

3. Participants consuming greater quantities of food items proposed to be beneficial in terms of AD risk (captured using the modified CSIROFFQ) have lower levels of blood-based biomarkers of inflammation, lower cerebral amyloid beta load (determined by Pittsburgh Compound-B positron emission tomography; PiB PET) and higher neuropsychological test scores compared to individuals consuming smaller quantities of these food items.

5.2. Results

5.2.1. Demographics of the validation study cohort

The descriptive statistics of the validation and 'reliability' study cohort are shown in Table 5.2. In summary, 49 individuals participated in the validation and 'reliability' study; of these, 32 were female and 17 were male. The participants had an average age of 72.2 ± 4.4 and an average body mass index (BMI) of 25.9 ± 3.3. Nearly 75% of the cohort was born in Australia and just over 25% had 12 or less years of education, whilst over 26% carry the *APOE* ε4 allele. When comparing the males and females following stratification of the cohort by gender, we saw that males were significantly older than females ($p = 0.046$; ANOVA) and as expected, had a higher BMI ($p = 0.028$; ANOVA; Table 5.2).

Table 5.2: *Descriptive statistics for the validation and 'reliability' study healthy control cohort as a whole, and subgroups following stratification of the cohort by gender.*

	Total Sample (n = 49)	Males (n = 17)	Females (n = 32)	*p*-values for gender differences
Age; y	72.2 ± 4.4	73.9 ± 4.5	71.3 ± 4.2	**0.046***
Gender; men, n (%)	17 (34.7)			
Country of birth; Australian, n (%)	36 (73.8)	14 (82.4)	22 (68.8)	0.305
Body mass index[i]; kg/m^2	25.9 ± 3.3	27.3 ± 4.0	25.2 ± 2.7	**0.028***
Presence of *APOE* ε4 allele; n (%)	13 (26.5)	5 (29.4)	8 (25.0)	0.746
Education ≤ 12 y; n (%)	13 (26.5)	7 (41.2)	6 (18.8)	0.311

Unless otherwise stated, data are presented as mean ± standard deviation of the mean. Bold indicates statistical significance ($p < 0.05$); Characteristics compared using independent samples t-test for continuous variables and χ^2 for categorical variables. [i]Body mass index is calculated as weight in kilograms divided by height in meters squared. Abbreviations: *APOE*, Apolipoprotein E; Kg, kilogram; m^2, meters squared; y, years.

5.2.2. Reliability results

It is important to determine if responses to a FFQ are reproducible or 'reliable', i.e. if individuals recall the same intake over the past year on different occasions. To investigate the 'reliability' of the modified CSIROFFQ we assessed the correlation of 207 food and beverage items or groups of items from two administrations of the modified CSIROFFQ, which were completed seven days apart, on average. Using Pearson's correlation coefficients we determined that seven of these 207 items were correlated at the $p < 0.05$ level, 24 were correlated at the $p < 0.01$ level and 154 were highly correlated at the $p < 0.001$ level. Twenty two items did not show correlation, these uncorrelated items were comprised of mostly individual fruits and vegetables, and takeaway foods for which low consumption levels were reported in both administrations of the FFQ. With respect to these uncorrelated items, the actual difference in the consumption level, determined using the two completed FFQs, ranged from 0.02 grams for chicken nuggets to 24.03 grams for milk. Pearson's correlation coefficients for all items assessed ranged from -0.181 for water or spring water to 1.000 for rum and soda and frankfurters or saveloys, with a mean of 0.595.

We then used an additional method to assess 'reliability' of the two administrations of the modified CSIROFFQ, namely intra-class correlation coefficients. Using this method, we found remarkably similar results; observing that 11 of the 207 items were correlated at the $p < 0.05$ level, 23 items were correlated at the $p < 0.01$ level and 148 items were highly correlated at the $p < 0.001$ level, leaving a remainder of 25 items for which intake did not show correlation (20 of these uncorrelated items were identical to uncorrelated items from Pearson's correlation coefficients above). The intra-class correlation coefficients for all items assessed ranged from -0.174 for water or spring water to 1.000 for rum and soda, with a mean of 0.544. Appendix 4 shows the Pearson's and intra-class correlation coefficients for this 'reliability' study.

Intake of herbs and spices reported following completion of the FFQ on two occasions was compared utilising binary scores assigned to 'yes' or 'no' answers to questions relating to whether an individual had consumed each of 19 herbs or spices in the last fortnight. Twenty six of the 49 participants involved in the study gave inconsistent answers in the two

administrations of the FFQ, i.e. they answered 'yes' to one or more of the questions during the first FFQ administration and 'no' during the second administration, or vice versa. In total there were 127 incorrect answers out of a total of 931 (19 questions x 49 participants) herb and spice questions asked (13.6%).

Intake of dietary supplements was also assessed using the modified CSIROFFQ. The intake of a total of 104 supplements was recorded in both administrations of the questionnaire, whilst intake of 24 supplements was reported only in one administration of the FFQ. Of the 24 supplements reported only once, 18 were consumed with a frequency of once a week or less, and six were consumed more than once a week. The final question concerning supplement intake within the modified CSIROFFQ is an open-ended question regarding intake of "any other supplements" not listed previously within the questionnaire (e.g. magnesium, iron, and probiotics). Seven supplements included in the response to this open-ended question were inconsistent between the two administrations of the FFQ, i.e. participants stated that they consumed a supplement during the first FFQ administration which was not included in the second administration, or vice versa (this open-ended question recorded intake of a total of 23 supplements during the two administrations of the questionnaire). In summary however, the overall results of the 'reliability' study suggest that the vast majority of the modified CSIROFFQ has a 'good' and 'adequate' degree of reproducibility with the greatest variance observed within the supplement intake questions.

5.2.3. Validation results

We used a four-day weighed food record to assess the validity of the questions modified or added to the online CSIROFFQ. Before commencing the validation study, participants attended an orientation session. During the orientation session, detailed verbal and written instructions regarding the completion of the four-day weighed food records (Sunday through to Wednesday) were provided. Digital scales weighing to the nearest gram, and measuring cups and spoons were also provided to each participant, as was a sample of a completed food record. Each participant was requested to record the weight of all food and beverages consumed during the four-day period (either at home or away from home) and to record brand names, methods of food preparation and ingredients of recipes in a

specifically designed diary (Appendix 3). Participants were additionally asked to record the amount of any leftover food at the end of a meal or snack consumed either at home or away from home, and to also report any dietary supplements consumed. All food records were reviewed face-to-face with the participant upon completion of the four-day period and unclear or missing information was clarified. Four-day weighed food records were analysed using FoodWorks 7 Professional version 7.0 (Xyris Software (Australia) Pty Ltd, Highgate Hill, QLD) and the AUSNUT 2007 database of Australian Foods, to yield grams per day of foods and beverages consumed. The grams per day of foods and beverages consumed as documented by the weighed food records was compared to the intake reported following completion of the first administration of the modified CSIROFFQ.

Due to the relatively small number of participants undertaking the validation study (n = 49), there were a small number of people consuming some dietary items (e.g. specific types of fish, berries, seeds, nuts, fats, and milk chocolate). For this reason, we grouped some items together for the purpose of validation (e.g. total fish, total seeds, total nuts, total berries, total grapes, total rice, total chocolate, and total fats), in addition to validating individual items wherever possible.

Pearson's correlation coefficients revealed that there was a high correlation ($p < 0.001$ level) between the recorded intake of seven of the added items according to the modified CSIROFFQ and the recorded intake of the same foods according to the four-day weighed food record (total fish, dark chocolate, total chocolate, total grapes, total seeds, total nuts and almonds), three added items were correlated at the $p < 0.01$ level (sesame seeds, white rice, and total rice) and two added items were correlated at the $p < 0.05$ level (olives and sunflower seeds). Pearson's correlation coefficients for all items that were validated ranged from -0.051 for red wine to 0.946 for cranberries (Table 5.3).

We then utilised intra-class correlation coefficients as a secondary method to assess validity: the results of the two methods were in general agreement. Using the second method we observed that six of the added items were highly correlated at the $p < 0.001$ level (total fish, total grapes, total rice, total seeds, total nuts and almonds), three dietary items were correlated at the $p < 0.01$ level (olives, dark chocolate, and total chocolate), and

three added items were correlated at the $p < 0.05$ level (sunflower seeds, white rice, and canola oil) when comparing intake determined by the modified CSIROFFQ and four-day weighed food record. The intra-class correlation coefficients for all items validated ranged from -0.040 for red wine to 0.945 for canola oil (Table 5.3).

Table 5.3: *Pearson's and intra-class correlation coefficients assessing similarity of intake of dietary items added to the modified CSIROFFQ compared to intake of these items determined using weighed food records.*

Food or beverage	Pearson's correlation coefficient	*p*-value	Intra-class correlation coefficient (F statistic)	*p*-value	Number of participants reporting consumption via the weighed food record
Total canned fish	0.157		0.117		14
Total fish	0.784	***	0.700	***	44
Seafood	0.227		0.125		5
Olives	0.847	*	0.838	**	6
Dark chocolate	0.814	***	0.717	**	13
Total chocolate	0.449	***	0.406	**	21
Red wine	-0.051		-0.040		16
White wine	-0.312		-0.281		11
Sparkling wine/Champagne	0.453		0.023		3
Total wine	0.263		0.200		23
Green tea	0.273		0.041		13
Black grapes	-0.265		-0.132		12
Green grapes	0.320		0.076		13
Total grapes	0.550	***	0.527	***	23
Pumpkin seeds	0.042		0.039		9

Sesame seeds	0.915	**	0.668		6
Sunflower seeds	0.831	*	0.799	*	6
Total seeds	0.896	***	0.621	***	14
Strawberries	-0.209		-0.195		9
Blueberries	0.620		0.449		7
Cranberries	0.946		0.753		7
Total berries	0.234		0.112		19
Walnuts	0.214		0.188		9
Almonds	0.802	***	0.718	***	23
Cashew nuts	0.563		0.431		10
Peanuts	0.397		0.342		9
Brazil nuts	-0.593		-0.469		10
Total nuts	0.448	***	0.429	***	33
White rice	0.694	**	0.512	*	15
Total rice	0.441	**	0.421	***	17
Sunflower oil	0.225		0.220		4
Olive oil	0.199		0.199		28
Extra virgin olive oil	0.813		0.650		5
Canola oil	0.945		0.945	*	7
Butter	0.126		0.085		23
Margarine	-0.185		-0.185		22
Total fats	0.148		0.146		47

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: CSIROFFQ, Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire.

Validation of herb and spice intake revealed that eight people gave at least one incorrect answer whereby they answered 'no' to herb and spice intake questions within the CSIROFFQ but documented consumption in the weighed food record; this resulted in a total of ten incorrect answers (1.1% of the possible 931 questions). By contrast, 42 individuals gave at least one incorrect answer whereby they answered 'yes' to herb and spice consumption questions within the CSIROFFQ but documented 'no consumption' via completion of the weighed food record; this yielded a total of 255 incorrect answers (27.4% of the possible 931 questions). It is important to note however, that the herb and spice questions in the modified CSIROFFQ ask "which of the following herbs or spices do you consume at least once a fortnight", whilst the weighed food record was completed over just four-days, it is possible therefore that these herbs or spices are consumed at least once a fortnight but their consumption was not captured by the weighed food record. Additionally, some specific herbs and spices in mixed dishes and drinks documented by completion of the weighed food records could not be determined, and hence, could not be included as 'consumed', for example spices in chai latte beverages.

Validation of supplement intake revealed that a total of 85 supplements were consumed by a total of 35 participants during the completion of the four-day weighed food record. Of these 85 supplements, 81 were included during completion of the modified CSIROFFQ (individually asked about, not including the final open-ended question in the FFQ; 95%), leaving four which were consumed during the four-day weighed food record but not stated as being consumed during completion of the modified CSIROFFQ (5%). By contrast, the modified CSIROFFQ recorded intake of a total of 119 supplements (captured using specific individual questions regarding the supplements) consumed by a total of 39 participants. Of these 119, 81 were included in the corresponding weighed food record (68%) and 38 were not included in the corresponding weighed food record (32%). Of the 38 that were not included, 33 were stated as being consumed weekly or less than weekly, therefore it is not surprising that their intake was not captured during the four-day weighed food record period. If we exclude these 33 infrequently consumed supplements, we observe 94% inclusion of supplement intake in both the CSIROFFQ and the weighed food records. For the remaining supplements which are not individually listed in the modified CSIROFFQ

but are instead included in the final, open-ended question, 27 supplements were consumed by 21 participants during the four-day weighed food record period. Of these 27 supplements, 16 (59%) were also included in the response to the final supplement question within the modified CSIROFFQ (the open-ended question which captures intake of "any other supplements not individually listed within the preceding questions"). In summary, the overall results of the validation study suggest that the modified CSIROFFQ can confidently be used to assess intake of at least 12 of the food and beverage items which were added to or modified within the online CSIROFFQ. The modified CSIROFFQ can also assess accurately the intake of herbs and spices, and supplements. However, intake of a number of items with generally low (or infrequent) consumption levels could not be validated with this sample size and length of weighed food record, and further work is required.

5.2.4. Relationship between consumption of foods of interest in Alzheimer's disease research added to the modified Commonwealth Scientific Industrial and Research Organisation food frequency questionnaire, cognition, blood-based biomarkers of inflammation and neuroimaging biomarkers of Alzheimer's disease

5.2.4.1. Construction of an 'Alzheimer's disease diet index'

The validation study described in section 5.2.3 revealed there were 12 items added to the modified CSIROFFQ for which intake, as determined by completion of the modified questionnaire, correlated significantly with the intake levels documented by completion of the four-day weighed food record (assessed using Pearson's correlation coefficients). These 12 items were divided into those for which consumption is proposed to be beneficial in terms of AD risk and those for which the contrary is suggested. Participants were assigned a value of 1 for each of the proposed beneficial items if their consumption was at or above the sex-specific median intake level (total fish, olives, dark chocolate, total grapes, sesame seeds, sunflower seeds, total seeds, almonds and total nuts). By contrast, participants were assigned a value of 1 for each of the proposed detrimental items if their consumption was below the sex-specific median intake level (white rice). Total chocolate and total rice were not incorporated in the construction of the 'AD diet index', as elements of these food items are expected to be beneficial in terms of AD risk (dark chocolate and brown rice) whilst

other elements are proposed to be detrimental with respect to AD risk (milk chocolate and white rice). The points were summed to produce a score ranging from 0 to 10, with a higher score proposed to be 'beneficial' in terms of AD risk. Table 5.4 lists the characteristics of the 'AD diet index'. Of the 49 individuals who participated in the validation study, and consequently for whom an 'AD diet index' was constructed, no one achieved an 'AD diet index' score of 0, 9 or 10. To further divide individuals into proposed 'higher' or 'lower' AD risk categories on the basis of 'AD diet index' scores, participants with a score of 1 – 4 were assigned a binary value of '1' to represent proposed 'higher' AD risk, and participants with a score of 5 – 8 were assigned a binary value of '0' to represent proposed 'lower' AD risk. These binary scores were then assessed cross-sectionally for associations with cognition, blood-based inflammation biomarkers and neuroimaging data collected as part of the Australian Imaging, Biomarkers and Lifestyle study (AIBL) of ageing.

Table 5.4: *Characteristics of the 'Alzheimer's disease diet index'*

'Alzheimer's disease diet index'
High intake of total fish
High intake of olives
High intake of dark chocolate
High intake of total grapes
High intake of sesame seeds
High intake of sunflower seeds
High intake of total seeds
High intake of almonds
High intake of total nuts
Low intake of white rice

5.2.4.2. 'Alzheimer's disease diet index' and cognition

A range of cognitive tests including Mini-Mental State Examination (MMSE), California Verbal Learning Test (CVLT) delayed recall, recognition and recognition discrimination, Rey Complex Figure Test (RCFT) 3 minute delayed recall, and Controlled Oral Word Association Task (COWAT) were carried out on the cohort (see section 2.10.4. for details of test administration and scoring protocols). The mean neuropsychological test scores were compared between the 'low' and 'high' AD risk groups, determined on the basis of 'AD diet index' adherence. Independent samples t-tests were initially used to compare mean neuropsychological test scores between the two AD risk groups, and this analysis was followed by general linear models in order to control for the most common confounding variables with respect to diet and cognition; i.e. age, gender, years of education, *APOE* ε4 allele carriage, country of birth, and BMI. All analyses were cross-sectional, with the data from the assessment time-point closest to the date of the validation study utilised in the analysis.

The independent samples t-tests revealed that the participants classified as proposed 'higher' risk for AD had significantly worse performance in the CVLT recognition discrimination test (a measure of verbal learning and memory) than those classified as 'lower' AD risk (T = 2.138, p = 0.038; Table 5.5). However, having controlled for the confounding variables listed above using general linear models, the significant difference did not remain (data not shown). Figure 5.1 shows unadjusted box plots for the six neuropsychological tests assessed following stratification of the cohort on the basis of 'AD diet index' into the proposed 'low' and 'high' AD risk groups.

Table 5.5: *Difference in mean neuropsychological test scores between the proposed 'high' and 'low' Alzheimer's disease risk groups stratified on the basis of 'AD diet index'; Independent samples t-tests.*

	Mean test score – 'low' AD risk group[a]	Mean test score - 'high' AD risk group[a]	T statistic	*p*-value	95 % CI
MMSE	29.0 ± 1.1	28.83 ± 1.2	0.502	0.618	-0.501 – 0.834
CVLT delayed recall	12.80 ± 2.3	11.17 ± 3.9	1.779	0.082	-0.214 – 3.418
CVLT recognition	15.52 ± 0.8	14.71 ± 2.1	1.830	0.074	-0.081 – 1.704
CVLT recognition discrimination	3.41 ± 0.4	3.01 ± 0.8	2.138	**0.038***	0.024 – 0.784
RCFT 3 minute delayed recall	20.42 ± 6.2	17.35 ± 5.1	1.893	0.065	-0.192 – 6.324
COWAT	43.56 ± 9.9	41.33 ± 10.7	0.757	0.453	-3.693 – 8.147

[a]data presented as mean ± standard deviation. Bold indicates statistical significance (*$p < 0.05$). Abbreviations: AD, Alzheimer's disease; CI, confidence interval; MMSE, Mini-Mental State Examination; CVLT, California Verbal Learning Test; RCFT, Rey Complex Figure Test; COWAT, Controlled Oral Word Association Task.

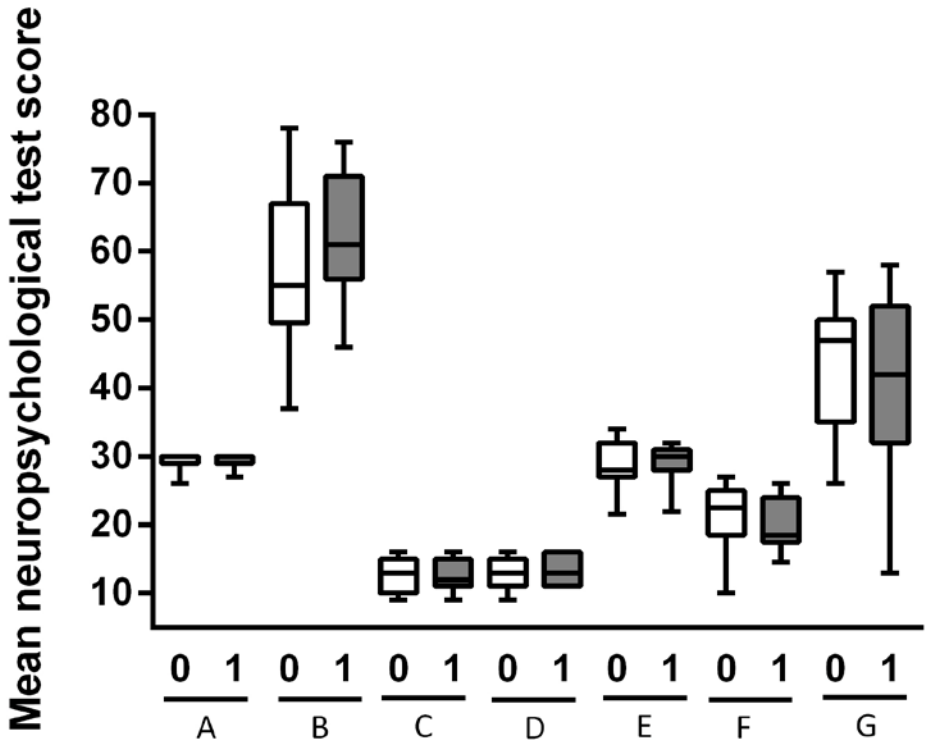

Figure 5.1: *Unadjusted box plots for the six neuropsychological tests following stratification of the cohort on the basis of 'AD diet index' into the proposed 'high' and 'low' AD risk groups.*

0 = proposed 'low' AD risk, 1 = proposed 'high' AD risk. Abbreviations: AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; CVLT DR, California Verbal Learning Test delayed recall; CVLT R, California Verbal Learning Test recognition; CVLT RD, California Verbal Learning Test recognition discrimination; RCFT 3D, Rey Complex Figure Test 3 minute delayed recall; COWAT, Controlled Oral Word Association Task.

5.2.4.3. 'Alzheimer's disease diet index' and blood-based biomarkers of inflammation

It is well established that there are links between inflammation, particularly chronic inflammation, and AD, and in Chapter 4, relationships between diet and inflammation were reported. Therefore, it was anticipated that differences may be found when comparing the mean levels of the blood-based inflammatory biomarkers measured by PathWest laboratories which were analysed in section 4.2.4.2. (haemoglobin, red blood cell count, erythrocyte sediment rate, platelet count, white cell count, neutrophils, lymphocytes, monocytes, eosinophils and basophils) between the proposed 'high' and 'low' AD risk groups (again determined on the basis of 'AD diet index'), using the same methods as those utilised in the cognition analysis reported in section 5.2.4.2. (i.e. independent samples t-tests followed by general linear models controlling for confounding variables). There were no significant differences in the mean levels of each of the blood-based inflammatory biomarkers between the proposed 'high' and 'low' AD risk groups as determined by independent samples t-tests (data not shown) and general linear models controlling for confounding variables (Table 5.6).

Table 5.6: *Difference in mean blood-based inflammatory biomarker levels between the proposed 'high' and 'low' Alzheimer's disease risk groups; General linear models.*

	Mean biomarker level - 'low' AD risk group[a]	Mean biomarker level - 'high' AD risk group[a]	F statistic	*p*-value
Haemoglobin	143.68 ± 10.5	139.53 ± 9.1	3.723	0.061
Red blood cell count	4.73 ± 0.4	4.58 ± 0.3	2.261	0.140
Erythrocyte sediment rate	12.83 ± 12.0	8.58 ± 5.8	1.958	0.169
Platelet count	198.36 ± 51.5	221.00 ± 38.6	2.163	0.149
White cell count	5.42 ± 1.1	5.91 ± 1.2	1.708	0.198
Neutrophils	3.21 ± 0.9	3.68 ± 1.1	2.414	0.128
Lymphocytes	1.57 ± 0.4	1.53 ± 0.3	0.431	0.515
Monocytes	0.45 ± 0.1	0.50 ± 0.2	0.446	0.508
Eosinophils	0.16 ± 0.1	0.17 ± 0.1	0.038	0.847
Basophils	0.04 ± 0.1	0.04 ± 0.0	0.435	0.513

Model includes age, gender, years of education, *APOE* ε4 allele carriage, country of birth (Australia vs. Other), and baseline body mass index as covariates. [a]data presented as mean ± standard deviation. Abbreviations: AD, Alzheimer's disease; *APOE*, Apolipoprotein E.

5.2.4.4. 'Alzheimer's disease diet index' and cerebral amyloid load

Thirty seven of the 49 participants included in the modified CSIROFFQ validation study had previously undergone a PiB PET scan as part of their routine AIBL study participation. Depending on an individual's PiB PET standardised uptake value ratio (SUVR) they were classified as either PiB PET negative (SUVR < 1.5; representative of lower levels of amyloid in the brain) or PiB PET positive (SUVR ≥ 1.5; representative of significant cerebral amyloid load). Multinomial logistic regressions were used to calculate the odds of being PiB PET positive rather than PiB PET negative if individuals were in the proposed 'high' AD risk group compared to the 'low' AD risk group determined on the basis of 'AD diet index' score. Interestingly, the odds of being PiB PET positive were 6.589 greater if an individual was in the proposed 'high' risk AD group compared to the 'low' risk AD group. However, this result lacked statistical significance (p = 0.070; Table 5.7), and was merely approaching significance, likely due to the small sample size.

Table 5.7 *Odds ratio of being PiB PET positive rather than PiB PET negative for individuals in the proposed 'high' AD risk group compared to individuals in the proposed 'low' AD risk group.*

Odds Ratio	Confidence Interval	*p*-value
6.589	0.860 – 50.508	0.070

Model includes age, gender, *APOE* ε4 allele carriage, years of education, country of birth (Australia vs. Other), and baseline body mass index as covariates. PiB PET negative = SUVR < 1.5, PiB PET positive = SUVR ≥ 1.5. Multinomial logistic regression analysis used to calculate odds ratios. Abbreviations: AD, Alzheimer's disease; *APOE*, Apolipoprotein E; PiB PET, Pittsburgh Compound-B positron emission tomography; SUVR, standardised uptake value ratio.

5.3. Discussion

The reproducibility and comparative validity of the online modified CSIROFFQ were assessed in this chapter by comparing data from the modified CSIROFFQ at two administrations (reproducibility) and by comparing food intake documented using four-day weighed food records with data generated from the first administration of the modified CSIROFFQ (validity). Over 85% percent of the 207 food and beverage items or groups of items assessed for reproducibility were significantly correlated between questionnaire administrations, as shown by Pearson's and intra-class correlation coefficients. The items lacking significant correlation were mainly takeaway foods, and individual fruit and vegetable items that were consumed at low levels. Thus, the overall reproducibility of the modified CSIROFFQ appears to be good.

Validity of an FFQ is the degree to which the instrument measures the diet of the subjects it was designed to study. Our study found that intake of a number of food groups and individual items that were added to the CSIROFFQ were significantly correlated between the weighed food record and the modified FFQ results including; total fish, total chocolate, dark chocolate, total grapes, total seeds, sesame seeds, sunflower seeds, total rice, white rice, olives, almonds and total nuts, suggesting validity of measurement of intake of these dietary items. We had a small sample size of 49 participants completing a four-day weighed food record, and we were attempting to validate foods of interest to AD research, which are not regularly consumed by the majority of people or are consumed in small amounts. For this reason, instead of validating all items individually, some items had to be summed to form food groups. Statistical analysis showed that the intake of the majority of these groups (determined using the weighed food record) correlated with intake data from the modified CSIROFFQ.

Of the 12 items added to the modified CSIROFFQ for which intake was significantly correlated with intake documented by completion of the four-day weighed food record, items were divided into those with proposed beneficial effect in terms of AD risk and those with proposed detrimental effect in relation to AD risk. Intake of each of the correlated items was used to construct an 'AD diet index' for every participant, and the index was

used to assign individuals to a proposed 'high' or 'low' AD risk group. The proposed 'high' and 'low' AD risk groups were then assessed cross-sectionally for associations with neuropsychological test scores, blood-based biomarkers of inflammation and cerebral amyloid load data collected as part of the AIBL study.

Independent samples t-tests revealed that the proposed 'high' AD risk group performed worse in the CVLT recognition discrimination test than the proposed 'low' AD risk group: However, these differences were no longer evident when confounding variables were controlled for. There were no differences in the levels of blood-based biomarkers of inflammation between the proposed 'high' and 'low' AD risk groups. Furthermore, there was no significant difference observed in the odds of being PiB PET positive when comparing the proposed 'low' and 'high' AD risk groups; the results were however trending towards significance ($p = 0.070$). We hypothesise that with a larger sample size an association where individuals proposed to be at 'higher' risk of AD (consume less beneficial foods) are more likely to be PiB PET positive (have higher cerebral amyloid load) may be observed.

These results further suggest that the questions added to or modified within the CSIROFFQ are valid and demonstrate utility in terms of assigning individuals to proposed 'lower' or 'higher' AD risk groups on the basis of their 'AD diet index' score. It is likely that a larger sample size and a longer duration of weighed food record (> four days) would strengthen the relationships observed. Additionally, a future aim is to validate the entire online modified CSIROFFQ, including nutrient and antioxidant intake.

Whilst the reference dietary method of choice for FFQ validation studies is reported to be weighed food records (259), ideally, a biochemical measure would provide an independent and objective assessment of nutrient intake: However, such measures are expensive and require the use of sophisticated laboratories. An additional future aim is further validation of the modified CSIROFFQ using a more direct measure of diet such as blood-based biomarkers.

It is also important to note the limitations of the results presented in this chapter. A general limitation of validation studies, including ours, is that results are not necessarily transferable to other populations due to regional variations in local foods (455). Furthermore, agreement between the two methods of dietary data collection utilised in this chapter may be affected by reporting of items in the FFQ which were not observed in the four-day weighed food record, or were consumed by few people (e.g. specific types of fish, seeds and berries). The day of the week may also influence the result of validation studies: In our study, the greatest proportion of records was collected on weekdays (75%) with the remaining 25% reflecting a weekend day, this division was deliberate as it is perceived that 'usual' dietary intake occurs during the week with greater variation observed at weekends (259, 454). Finally, an additional limitation of weighed food records is the fact that participants may have changed their diet on the day's intake was recorded in an attempt to make the process easier for themselves, despite requests that participants consume their normal diet and reassurance from all that this was the case.

Irrespective of the limitations described above, the present study provides initial evidence of the reliability of the modified CSIROFFQ and of its validity as an alternative measure of dietary intake. The good level of agreement observed between responses and with respect to the weighed food records, suggests the modified CSIROFFQ is repeatable and shows a good level of validity, and thus can provide an acceptable assessment of long-term dietary intake in Australian older adults. Furthermore, the modified CSIROFFQ appears to make an important contribution to the tools available for assessing usual dietary intake in Australian older adults with respect to AD research.

Chapter Six

General discussion and future directions

6.1. Introduction

Alzheimer's disease (AD) is the most common form of senile dementia and currently affects over 35 million people worldwide. Intracellular neuritic plaques which are composed mainly of amyloid beta (Aβ) peptide aggregates, and intracellular neurofibrillary tangles, which consist of abnormally hyper-phosphorylated tau, are the pathological hallmarks of AD, in the brain. These are believed to contribute to synaptic dysfunction and neuronal death, although small oligomers of the Aβ peptide are thought to be the more toxic form of the aggregated forms of Aβ peptide. Currently, definitive diagnosis of AD relies upon the presence, at autopsy, of these pathological hallmarks, in specified amounts in discrete regions of the brain. To date, there is no cure available for AD, and for this reason much attention has been directed to identifying factors which may delay onset or slow disease progression. We now know that the neuropathological changes associated with AD precede onset of symptoms by 15 – 20 years, thereby presenting a 'window of opportunity' to modify disease course. It is clear therefore that there is significant benefit to ascertaining which lifestyle factors may protect against or contribute to the development of AD. Identification of such factors might permit early intervention to modify disease course, enabling delay or ideally prevention of onset of AD (11). Diet modification represents one potential intervention strategy accessible to all which is also economically advantageous over medical interventions with reduced incidence of adverse side effects.

Individuals consume diets that contain both nutrient and non-nutrient substances rather than single foods. Consequently, misleading conclusions on the effect of consumption of a single nutrient, food or dietary component on health outcomes can be drawn. It may be more useful therefore to examine indices of food and nutrient intake that express several related aspects of diet concurrently rather than focus on consumption of single nutrients (280): Consequently, dietary pattern analysis has emerged (219).

The present thesis aimed to further the understanding of the association between diet and AD. To achieve this aim, four dietary patterns were constructed using data collected via administration of the Cancer Council of Victoria Food Frequency Questionnaire (CCVFFQ), namely; Mediterranean diet (MeDi), inflammatory dietary index, prudent diet and western diet patterns. The relationship of adherence to these dietary patterns, cognition and cognitive decline was assessed in the large, well-characterised elderly Australian Imaging, Biomarkers

and Lifestyle Study of Ageing (AIBL) cohort. The potential mechanisms underlying the observed effects of dietary pattern adherence on cognitive decline were investigated in this cohort using blood-based and neuroimaging biomarker data collected as part of the AIBL study. Additionally, by adding questions regarding intake of foods of interest in AD research to the online Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire (CSIROFFQ), we aimed to develop and validate an AD-specific food frequency questionnaire to be utilised for future research into dietary factors and AD risk.

6.2. Summary of main results

The main results from this thesis are as follows:

- AD participants in our cohort demonstrated reduced adherence to the MeDi and prudent diets, and greater adherence to the western diet and the inflammatory dietary index (equating to consumption of less anti-inflammatory foods) compared to healthy controls. Further, healthy control participants demonstrated greater adherence to the MeDi and prudent diets, and reduced adherence to the inflammatory dietary index (consumption of more anti-inflammatory foods) compared to individuals with mild cognitively impairment (MCI).

- There were no cross-sectional associations between adherence to the four dietary patterns and global cognition or any of the six composite cognitive domains assessed.

- Longitudinally, there were no gender-dependent associations between adherence to the four dietary patterns and global cognition or any of the six composite cognitive domains assessed.

- Higher baseline adherence to the ‘healthy’ MeDi was associated with decreased decline in the executive function composite cognitive score 36 months later in Apolipoprotein E *(APOE)* ε4 allele carriers only.

- Higher baseline adherence to the ‘unhealthy’ western diet was associated with increased decline in the visuospatial functioning composite cognitive score 36 months later in *APOE* ε4 allele non-carriers.

- Dietary pattern adherence appeared to have no effect on the rate of transition from healthy control clinical classification at baseline to mild cognitive impairment (MCI) or AD status after 36 months in the cohort as a whole and following stratification of the cohort by gender and *APOE* ε4 allele carriage (although this analysis was likely limited by low numbers of individuals transitioning over this period of time).

- Whilst we report some associations between dietary pattern adherence and blood-based biomarkers of metabolic syndrome and cardiovascular disease (CVD), our lack of significant associations between the MeDi and western diet patterns and our metabolic syndrome and CVD indexes as well as the individual components of the indexes, suggests that modulation of metabolic syndrome or CVD risk may not be the primary mechanisms underlying the reported effects of diet on cognition.

- We found our 'unhealthy' western dietary pattern to be positively associated with levels of blood-based biomarkers of inflammation and our 'healthy' MeDi and prudent diet patterns to be negatively associated with levels of blood-based biomarkers of inflammation. We also found our inflammatory dietary index to be strongly positively correlated with the levels of numerous blood-based biomarkers of inflammation, i.e. as intake of anti-inflammatory foods decreased (indicated by a higher inflammatory dietary index), the levels of blood-based biomarkers of inflammation increased. We observed many of these associations in both males and females as well as in *APOE* ε4 allele non-carriers and carriers. Our results suggest that the interplay between diet and elevated chronic inflammation may contribute to the observed effects of diet on cognition.

- Our preliminary results suggest that the modified CSIROFFQ is reliable and valid as a method of capturing dietary intake of foods of particular interest for AD research.

6.3. Dietary patterns and cognitive decline

Chapter 3 provided cross-sectional evidence that AD participants in our cohort consume fewer foods in the MeDi and prudent diet (high in fruits, vegetables and nuts), less anti-inflammatory foods, and more foods in the western diet (high in red and processed meats, chips, refined grains, potatoes, sweets and condiments) compared to cognitively healthy

controls. However, the nature of this analysis means that the impact of AD diagnosis on dietary habits cannot be excluded.

Cognitive decline is the progressive loss of cognitive functions, including memory, and may lead to dementia of which AD is the most common type, accounting for 60 - 80% of cases (279). Longitudinal results from Chapter 3 included only those individuals classified as cognitively healthy at baseline to circumvent the potential confounder of AD diagnosis described above. The longitudinal results highlighted the detrimental nature of a western diet and proposed the importance of adhering to a 'healthy' dietary pattern such as the MeDi, with respect to reducing risk for cognitive decline over 36 months, with executive function and visuospatial functioning seemingly most susceptible to the influence of diet. Our results suggest that there are differential effects of diet on cognition that are likely to be contingent on *APOE* ε4 allele carriage; the MeDi effects were observed only in *APOE* ε4 allele carriers and the western diet association with increased cognitive decline was observed only in *APOE* ε4 allele non-carriers.

Results from previous studies on the association between dietary patterns and cognitive decline are inconsistent. Several American and European studies have shown a positive relationship between adherence to the MeDi and slower cognitive decline or reduced AD risk (1-7). By contrast, other studies have concluded that higher consumption of the MeDi was not protective against cognitive decline (226, 282, 283). Studies examining risk of cognitive decline using '*a posteriori'* developed dietary patterns are limited in number: these studies have generally found that higher consumption of an 'unhealthy' dietary pattern, rich in processed foods (similar to our western diet), is associated with increased cognitive decline with the converse true for a 'healthy' or 'whole food' (similar to our prudent diet) dietary pattern (6, 7). To our knowledge, an investigation into associations between dietary patterns and risk of AD or cognitive decline, looking specifically at the anti- or pro-inflammatory nature of dietary components, has not been attempted previously.

Whilst it was evident from our analysis that dietary pattern adherence played an important role in cognitive decline, we did not observe an association between dietary pattern adherence and transition from healthy control clinical classification at baseline to MCI or

AD status after 36 months. It is worthy of note however, that this analysis was likely limited by low numbers of individuals transitioning over the period of follow up (n = 25; 4.7%), and additional longitudinal data collected from our cohort will further clarify the relationship between adherence to our dietary patterns and AD risk. To our knowledge, this is the first study extensively comparing MeDi, inflammatory dietary index, western and prudent diet pattern adherence to cognitive decline in an elderly, well-characterised cohort.

6.4. Mechanisms of action of dietary patterns

Chapter 4 explored potential mechanisms that might underlie the effects of diet on cognition reported in Chapter 3 by assessing the relationship of the dietary patterns with blood and neuroimaging biomarker data collected as part of the AIBL study. Our investigations focussed on factors that are well-established contributors to AD risk i.e. metabolic syndrome, CVD, and inflammation. We also assessed the relationship between the dietary patterns and magnetic resonance imaging (MRI)-determined brain volume and Pittsburgh Compound-B positron emission tomography (PiB PET)-determined cerebral amyloid load. A study of this comprehensive nature has, to our knowledge, not previously been undertaken.

As described earlier, the results of Chapter 3 revealed beneficial effects of MeDi adherence and detrimental effects of western diet adherence on cognition over 36 months. In Chapter 4, we reported associations between dietary pattern adherence and biomarkers of metabolic syndrome and CVD. However, our lack of significant associations between the MeDi and western diet patterns and our metabolic syndrome and CVD indexes as well as the individual components of the indexes, suggests that modulation of metabolic syndrome or CVD risk may not be the primary mechanisms underlying the effects of diet on cognition reported in Chapter 3 of this thesis.

Consistent with published literature, we found our ‘unhealthy’ western dietary pattern to be positively associated with levels of blood-based biomarkers of inflammation and our ‘healthy’ MeDi and prudent diet patterns to be negatively associated with levels of blood-based biomarkers of inflammation. We also found our inflammatory dietary index to be strongly positively correlated with the levels of numerous blood-based biomarkers of inflammation, i.e. as intake of anti-inflammatory foods decreased (indicated by a higher

inflammatory dietary index), the levels of blood-based biomarkers of inflammation increased: A relationship that has never before been investigated in a well characterised ageing cohort. We observed many of these associations in both males and females as well as in *APOE* ε4 allele non-carriers and carriers.

The strong associations between dietary pattern adherence and biomarkers of inflammation reported in Chapter 4 suggest that the interplay between diet and elevated chronic inflammation may contribute to the effects of diet on cognition described in Chapter 3 of this thesis. This hypothesis certainly warrants further investigation and it will become increasingly important to understand the 'pro-inflammatory' versus 'anti-inflammatory' contribution of each of the four dietary patterns investigated to further clarify whether inflammation is the primary candidate mechanism underlying the reported effects of diet on cognition.

Chronic inflammation processes play an important role in the pathogenesis of AD (456) and are considered a propagating factor for neurodegeneration, with severity of neuroinflammation correlating with the degree of tissue loss and cognitive decline. Cytokines and chemokines are important mediators of inflammation. The identification of interleukin 1β (IL1β), tumour necrosis factor α (TNFα), interleukin 6 (IL6), monocyte chemo attractant protein 1 and macrophage inflammatory protein 1β in senile plaques and associated microglia has further highlighted the importance of inflammation in AD (457, 458). Holmes *et al*. (459) reported that presence of both acute (manifestation of systemic inflammatory events over six months) and chronic inflammation (high TNFα level at baseline) is associated with a ten-fold increased rate of cognitive decline compared with individuals with low levels of TNFα at baseline and no systemic inflammatory events over the following six months. The insoluble Aβ and neurofibrillary tangles which are likely to be the stimuli for inflammation are present from preclinical stages of AD; thus up-regulation of complement, cytokines and other inflammatory mediators are likely present and chronically elevated from the same stage (324). Accumulating literature also supports the notion of diet influencing levels of inflammation. There is evidence to suggest dietary fatty acids can modulate markers of inflammation (460-463). Consumption of a diet high in trans fatty acids has been associated with higher C-reactive protein (CRP) concentrations and consumption of a diet high in oleic acid has been associated with lower IL6

concentrations (460). Consumption of a high fat western diet has been shown to result in increased astrocyte reactivity but no change in cognition, microglial reactivity or cytokine levels, however a very high fat lard diet resulted in impaired cognition, increased expression of the cytokines TNFα, IL6, monocyte chemotactic protein 1 and increased reactive astrocytosis and microgliosis (461). Dietary fish oils suppress production of pro-inflammatory cytokines in animal and human studies. Fish oil feeding has also been shown to ameliorate the symptoms of chronic inflammation in animal models and protect against inflammatory challenges (463). Individuals in a higher MeDi adherence tertile have been shown to possess on average a 20% lower CRP level, a 17% lower IL6 level, a 15% lower homocysteine and a 14% lower white cell count compared to those in a lower adherence tertile. Furthermore, Gu *et al.* (227) also found higher adherence to the MeDi was associated with lower levels of CRP. Fung *et al.* (296) used an alternate MeDi (this alternate MeDi excludes dairy, has a nut group, has only red and processed meat in the meat section and excludes potatoes) and concluded that individuals in the top adherence quintile compared to the bottom quintile had a 24% lower CRP concentration. A protective effect of adherence to the MeDi has also been reported by Panagiotakos *et al.* (297); for each unit of increasing adherence to the MeDi, there was a reduction of 3.1% in the average CRP level and 1.9% reduction in the average IL6 level. Moreover, an intervention study with 90 participants following a MeDi and 90 following a prudent diet found that the participants who consumed the MeDi had significantly reduced serum concentrations of CRP, IL6, IL7 and IL18 as well as decreased insulin resistance compared to those consuming the prudent diet (231). However, not all studies have found positive results for these inflammatory biomarkers: Dai *et al.* (331) found higher adherence to the MeDi was associated with reduced levels of IL6 but had no significant effect on CRP.

A ‘healthy' dietary pattern (similar to our prudent diet) has also been inversely related to plasma concentrations of CRP and vascular cell adhesion molecule 1. The authors additionally reported that western diet score was positively related to serum amyloid A and IL6 ($p < 0.001$; 300). Fung *et al.* (212) also found significant positive correlations between western dietary pattern adherence and insulin, CRP, leptin and homocysteine concentrations, and an inverse correlation with plasma folate concentrations. The authors’ prudent dietary pattern was by contrast positively associated with plasma folate and inversely correlated with insulin and homocysteine concentrations. Furthermore, Nettleton

et al. (299) derived four dietary patterns using factor analysis: The fats and processed meats pattern was positively associated with CRP, IL6 and homocysteine. The beans, tomatoes and refined grains pattern was positively related to intercellular adhesion molecule 1 (ICAM1). The whole grains and fruit pattern was inversely associated with CRP, IL6, homocysteine and ICAM1, and the vegetables and fish pattern was inversely associated with IL6.

As previously mentioned, in Chapter 4 we reported positive associations between MeDi and prudent diet adherence and blood-based biomarkers of inflammation and by contrast, negative associations between western diet and inflammatory dietary index and these biomarkers of inflammation. Whilst inflammation is a strong candidate mechanism to explain the effects of diet on cognition reported in Chapter 3, it is likely that a complex interplay exists with overlap of the effects of diet on several mechanisms concurrently. For example, the strongest results in Chapter 3 of this thesis were related to the beneficial effects of MeDi adherence on cognition. However, the MeDi did not appear to be highly correlated to any of the potential mechanisms investigated in Chapter 4. It may be that the MeDi exerts its protective effects via independent mechanisms not investigated in this thesis, or perhaps more likely is the possibility that rather than one pronounced mechanism of action, the MeDi acts via multiple mechanisms of (weaker level) action which synergistically confer protection: Both are plausible explanations that warrant further investigation.

Finally, whilst no significant associations were found between dietary pattern adherence, MRI-determined brain region volumes and PiB PET-determined cerebral amyloid load, several associations were approaching statistical significance. It is important to note that only a subgroup of participants underwent neuroimaging thereby reducing the sample size from 527 to 123. This reduced sample size is likely to have limited the power to observe associations of dietary pattern adherence with neuroimaging outcome measures. Consequently, we believe the relationship between diet and neuroimaging biomarkers of AD to be worthy of further investigation: An aim which can be achieved in our cohort in the near future courtesy of collection of additional longitudinal data and supplementary cross-sectional data from new study participants.

6.5. The effect of the Apolipoprotein E ε4 allele

Our results suggest that there are differential effects of diet on cognition that are likely to be contingent on *APOE* ε4 allele carriage. Compared to the *APOE* ε2 and ε3 isoforms, *APOE* ε4 is a less efficient cholesterol transporter (303). The management of cholesterol levels as well as high density lipoprotein: low density lipoprotein ratios by fish oils in specific diets, like the MeDi, has been proposed to prevent and treat the negative effects of the *APOE* ε4 genotype (304). Studies of *APOE* ε4 allele target replacement mice have shown a positive influence of fish oil-containing diets on behavioural and cognitive performances (305). A possible explanation for the MeDi effects on cognition being observed only in *APOE* ε4 allele carriers in our study is that the cholesterol transport non-carriers may already be functioning at a reasonable level due to their ε2/ε3 genotype, so MeDi components are not able to exert a statistically significant improvement.

Throughout our investigations of the effects of dietary pattern adherence on blood-based biomarkers of inflammation, we also observed associations that were contingent on *APOE* genotype. For example prudent diet was negatively associated with eosinophil level in *APOE* ε4 allele carriers, and inflammatory dietary index was positively associated with white cell count in *APOE* ε4 allele non-carriers, cross-sectionally at both the baseline time-point and 36 month follow up.

It has previously been shown that the apoE protein and the *APOE* genotype modulate the immune responses that are evident in most neurological diseases, including AD (464, 465). For example, mice that lack an *APOE* gene and subsequently, apoE protein demonstrate a large increase in pro-inflammatory cytokines, decreased resistance to infection and increased mortality; results which led the authors to suggest that the presence of apoE regulates macrophage function and affects overall immune status (464, 466, 467). Furthermore, microglia derived from *APOE* ε4/ε4 targeted replacement mice demonstrate a pro-inflammatory phenotype that includes altered cell morphology, increased nitric oxide production associated with increased nitric oxide synthase 2 messenger ribonucleic acid, and higher pro-inflammatory cytokine production (TNFα, IL6, interleukin 12 homodimer) compared to microglia derived from *APOE* ε3/ε3 targeted replacement mice. The effect is gene dependent and increases with the number of *APOE* ε4 alleles (464).

Ours is not the first study to report differential effects of lifestyle factors on aspects of cognitive decline and AD-related pathology that are contingent on *APOE* ε4 allele carriage. Notably, Brown *et al.* (132) showed that brain and blood Aβ levels are modulated by physical activity by *APOE* genotype-dependent mechanisms. Specifically, the authors reported that high physical activity is associated with lower plasma $A\beta_{1\text{-}42/1\text{-}40}$ in *APOE* ε4 allele non-carriers, while higher exercising *APOE* ε4 allele carriers had lower levels of brain amyloid. Brown *et al.* concluded that physical activity appears to attenuate the high levels of brain amyloid associated with *APOE* ε4 carriers, and the apparent lack of effect on plasma Aβ levels may reflect poor clearance of Aβ in the periphery in individuals with the *APOE* ε4 allele. Taken together, the results of Brown *et al*. and the findings reported in this thesis suggest that in future the assessment of lifestyle in the context of *APOE* genotype may help the development of strategies tailored to the needs of *APOE* ε4 allele carriers in the Alzheimer population who have the poorest prognosis with current treatment strategies. To further assess the relationship between nutrition and AD risk, a validated, AD-specific food frequency questionnaire is required.

6.6. Validation results

Accurate assessment of *overall* dietary intake is crucial to understanding the association between diet and chronic diseases: incorrect information may lead to false associations between dietary factors and diseases. There is a need to develop and validate methods of assessing food and beverage intake to facilitate the development of guidelines for the prevention of diet-related chronic disease. However, dietary questionnaires need to be both specific to the population being analysed, and to the disease of interest: currently, there is no AD-specific FFQ available.

From the literature, we identified foods of interest in AD research; included were pomegranates, nuts and seeds, herbs and spices, supplements, green tea, red wine, chocolate and oils. Intake of some of these items was already captured by completion of the existing online CSIROFFQ, however specific details regarding quantity, frequency, and/or subtypes of items were not collected, whilst information regarding intake of other items was entirely missing.

In an attempt to develop a questionnaire specific to AD research, we altered or added questions to the online CSIROFFQ relating to the foods of interest listed above. Chapter 5 of this thesis assessed the 'reliability' (if individuals recall the same intake over the past year on different occasions) and 'validity' (this ensures dietary questionnaires estimate true food intake) of the online modified CSIROFFQ. Our results showed 'good reliability' between dietary intake captured following two administrations of the FFQ, and a 'good level of agreement' between intake of food and beverage items captured by both the modified CSIROFFQ and a four-day weighed food record (a 'gold standard' of collection of dietary data for the purposes required by this study).

We further investigated the validity of the modified CSIROFFQ by assigning participants to a proposed 'high' or 'low' AD risk group depending on consumption of the validated foods of interest in AD research. We then investigated the difference in mean neuropsychological tests scores, mean inflammatory biomarker levels and cerebral amyloid load between these proposed 'high' and 'low' AD risk groups. Although none of the results reached statistical significance, several were trending towards an association, in particular increased likelihood of PiB PET positivity (representative of significant cerebral amyloid load) among the proposed 'high' risk AD group compared to the 'low' risk AD group. The small sample size may account for the lack of statistically significant results observed: The validation study was conducted on 49 cognitively normal participants and only 37 of these individuals underwent neuroimaging.

Together, these results suggest that the modified CSIROFFQ is 'reliable' and has an acceptable level of validity, therefore it is a tool which can provide an acceptable assessment of long-term dietary intake in Australian older adults, particularly in the context of AD research. However, to further confirm 'validity' we require an increased sample size, preferably with validation of nutrient status also confirmed using blood-based biomarkers.

6.7. Limitations

This thesis reports novel associations between diet and a number of variables associated with the development of AD. However, these findings should be interpreted within the context of the limitations of this study. The study utilised a relatively short follow-up period (36 months) for longitudinal analyses. As dietary intake is a long-term habit

associations between nutrition, cognitive function and blood- and brain-based AD biomarkers may be more clearly determined in studies of extended follow up. Seasonal variations in diet should also be considered in such studies.

The AIBL study cohort is highly educated and mostly Caucasian (> 90%); factors which may impact upon the translatability of our results to the wider community. In an attempt to counteract the effects of surveying such a homogenous group, the neuropsychological battery contained a large selection of cognitive tests that are likely to be sensitive to cognitive differences, even in such a high performing cohort. The ethnic 'make up' of the AIBL cohort however, may limit inferences regarding similar effects in other populations.

The FFQs used in this thesis rely on participants' estimations of food intake over the previous 12 month period; this results in a limitation common to studies of diet and disease, namely misclassification of exposure due to limited accuracy. This point is particularly salient among AD participants; we are relying on participants' carers for validation of answers, or assistance with completion of the FFQ, and therefore assuming the carers are cognitively intact themselves. Furthermore, misclassification of diet is not equal between persons with and without cognitive impairment, and this raises the potential for 'differential' misclassification bias. One methodological approach which partly circumvents the issue, and which we have employed in our longitudinal analysis, is inclusion of only individuals classified as healthy control at baseline (these individuals may or may not convert to MCI or AD at subsequent follow ups). To further reduce the likelihood of misclassification of exposure, any reported energy intake that was greater than 3500 kilocalorie (kCal) in women and 4000 kCal in men or lower than 500 kCal in women and 800 kCal in men was removed from the dataset. An additional limitation is the fact that the CCVFFQ is known to under-report intake of certain foods, including soft drinks and snack foods. We recognise that ideally a nutrient biomarker panel that reflects dietary intake would be employed as an additional approach to circumvent the limitations associated with using the FFQ; although this approach has its own limitations, i.e. a biomarker panel cannot be as exhaustive as to include every possible nutrient in food.

Two types of dietary patterns have been used in this thesis, '*a priori'* and '*a posteriori'*. 'A *priori'* patterns such as the MeDi and inflammatory dietary index reflect adherence to

specific dietary patterns or guidelines, and therefore results can only be as good as these underlying guidelines. The availability of dietary guidelines is required to define dietary indices, and generally the guidelines are not disease specific, hence adherence may reduce the risk of some diseases but not others. Dietary indices need to be updated as dietary recommendations for the population being analysed are modified, a process of continual revision. Limitations of '*a posteriori'* methods include the fact that they are exploratory in nature (281) and are based on complex statistical analyses that require investigator-led selection of a limited number of components to summarise the food patterns (243).

Moreover, the MeDi score itself has limitations; it weighs equally the underlying individual food categories, which in turn are composed of different numbers of food constituents. Further, our study was conducted in an Australian cohort, which is unlikely to adhere strictly to a diet typical of Mediterranean countries. Therefore, "true MeDi" adherence in our population may be significantly lower when compared with Mediterranean populations, and subjects with high MeDi adherence in Australia may be potentially categorized as low MeDi adherence subjects if compared with Mediterranean populations. However, our results support the notion that the beneficial effects of the MeDi are transferable to different populations.

It is also possible that diet is related to socioeconomic status or to other habits or characteristics related to better health and a lower risk for AD. We attempted to address this issue by adjusting for country of birth (Australia vs. Other), years of education, and body mass index, in all our analyses. Another confounder usually considered in nutritional analysis is caloric intake. We used caloric intake adjusted residuals for MeDi score calculation and also included caloric intake as a covariate in all analyses (as Willet and colleagues recommended; 468). Additionally, within the AIBL study we do not collect data pertaining to family origin, although we acknowledge the influence family origin will likely have on a participant's current dietary habits (469).

Despite the limitations discussed above, many additional aspects of our study provide confidence in our findings. The dietary data reported in Chapters 3 and 4 was collected using an instrument previously validated in earlier epidemiological studies (450), and we have taken a very conservative approach by controlling for a wide range of demographic variables, including age, years of education, and country of birth and using a *p*-value of

0.01 to determine statistical significance throughout this thesis. Furthermore, the AIBL study is renowned for its well-characterised cohort and for the high quality of data collected as part of the study (e.g. high quality neuropsychological data and high quality rigorously processed blood samples).

6.8. Future directions

If additional funds and time were available, we would ideally expand on the validation study results reported in Chapter 5 of this thesis by increasing the sample size, and collecting blood samples from participants in order to validate the CSIROFFQ with respect to blood-based biomarkers of nutritional intake.

Additional time would also allow inclusion of data collected from further follow-up assessment time-points into the cognitive and mechanistic analysis described in Chapters 3 and 4. Thereby providing a larger number of individuals 'transitioning' from healthy control status to MCI or AD and more thorough subsequent investigation of the association of dietary pattern adherence and diagnostic conversion rate. This additional follow-up data would also enable longitudinal expansion of our mechanistic investigations.

The field of AD biomarkers is ever-growing, thus, there are some potential biomarkers of AD that were not included in the current study that would be worthy of inclusion in future research. Further work would ideally include measurement of blood-based biomarkers of inflammation such as S100 calcium binding protein B (a potential biomarker of tissue damage), α1 anti-chymotrypsin (some studies suggest this protease inhibitor enhances the production of Aβ fibrils), and chemokines including microphage colony-stimulating factor. Other biomarkers that should be investigated include markers of oxidative stress such as some products of lipid peroxidation (F_2-isoprostanes) and oxidatively modified deoxyribonucleic acid. These biomarkers may provide further insight in relation to the link between diet and AD risk. The measurement of tau and Aβ levels in cerebrospinal fluid (CSF), combined with PiB PET standardised uptake value ratio (SUVR) for the determination of cerebral Aβ load, have become 'gold standards' in the context of AD biomarker research, with changes in these markers predictive of cognitive decline in healthy older adults (470). Whilst cerebral Aβ load as determined by PiB PET neuroimaging was incorporated into this thesis, unfortunately CSF measurements of Aβ

and tau were unavailable, thus presenting an additional possible avenue for investigation in the future. Analysis of PiB PET data collected following recent expansion of the neuroimaging cohort in addition to further longitudinal assessment would also be possible if more time were available.

Through the establishment of intervention trials, with numerous outcome variables including changes in levels of the biomarkers discussed above, the effects of diet on the development of AD neuropathology may become clearer. Tailoring of such intervention trials using specific trial groups (e.g. different components of the MeDi, supplements, with/without physical activity etc.), may also enable elucidation of dietary components that are likely to be most beneficial to cognitive health. Furthermore, such trials would also be able to investigate the effect of *APOE* ε4 allele carriage on these associations: For such investigations, an over-selection of people with this genotype is required. Although it is not possible to eradicate all confounding factors, such studies will provide more conclusive results. The collective evidence linking diet to AD risk needs to be recognised, and implementation of dietary advice favouring a healthy ageing brain in a clinical setting is a necessary 'next step' in this field of research.

6.9. Conclusions

The main findings reported in this thesis indicate that high consumption of a 'healthy' dietary pattern incorporating the specific components included in the MeDi, and low consumption of an 'unhealthy' dietary pattern like the western diet pattern has positive implications for reducing cognitive decline, with executive function and visuospatial functioning seemingly most susceptible to the effects of diet. Our mechanistic investigations suggest that interplay between diet and elevated chronic inflammation may contribute to the effects of diet on cognition described in this thesis. However, whilst inflammation is a strong candidate mechanism, it is likely that a complex interaction exists with overlap between the effects of diet on several mechanisms concurrently.

It is increasingly clear that the relationship between diet and brain health is complicated. The results presented in this thesis contribute to the quest for greater understanding of the role of nutrition as a protective factor against AD. This thesis also contributes to the

provision of a validated tool which can be used to further investigate dietary factors specific to AD.

With the high social and financial cost of AD poised to place considerable burden on Australian society in the coming decades, and with no cure or effective treatment currently in sight, it is essential that factors such as diet which may help to prevent or delay cognitive decline and AD are characterised, and observational studies such as AIBL provide a forum for this work. Beyond this however, there is a need to extend such knowledge to interventional research to determine a cause and effect relationship and thereby enhance the implementation and type of prevention programs involving diet. If successful, such interventional programmes will impact strongly and positively on both social and economic aspects of caring for the aged and may contribute to the development of 'Best Practice' preventative public health strategies to delay AD onset and reduce incidence in our ageing population.

Moreover, our assessment of dietary pattern adherence in the context of *APOE* genotype also suggests that a 'one size fits all' approach to nutrition is unlikely to be effective in terms of modulating AD risk. Further investigation of the effects of diet on cognition and AD-related pathology in the context of genotype may assist in the development of dietary strategies tailored to the needs of those who are at greater genetic risk of developing AD.

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Appendix 1

Participant information and consent document

PARTICIPANT INFORMATION AND CONSENT FORM

Version 16: 1st September 2012

STUDY EXTENSION

Title of Study:The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

Principal Researcher: Professor Ralph N. Martins

Associate Researchers: Dr. Hamid Sohrabi, Professor David Ames, Dr. Kathryn Ellis, Professor Colin Masters, Dr. Trevor Lockett, Professor Edmond Chiu, Dr. Olga Yastrubetskaya, Dr. Mathew Samuel, Ms. Kristy Draper, Mr. Kevin Taddei, Mr. Mark Rodrigues, Ms. Belinda Brown, Ms. Tania Taddei, Ms. Samantha Gardener, Ms. Rebecca Lachovitzki, Dr. Tejal Shah, Ms. Georgia Martins, Dr. Simon Laws, Dr. Veer Gupta, Dr. Stephanie Rainey-Smith, Dr. Andrea Wilson, Ms Kim Lucy Do, Ms Sabine Matthaes, Ms Rachel Buckley.

This Participant Information and Consent Form is 16 pages long. Please make sure you have all the pages.

1. YOUR CONSENT

You are invited to continue your participation in an extension to this research study of Alzheimer's disease.

This Participant Information document contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part in it.

Please read this Participant Information carefully. Feel free to ask questions about any information in the document. Should you have questions about the study you may contact:

Professor Ralph Martins (Principal Researcher):
Phone No. 0404-839-305 (all hours)
Dr Hamid Sohrabi (Registered Psychologist):
Phone No. 0404-418-270 (all hours)
Dr Stephanie Rainey-Smith (Study Co-ordinator)
Phone No. 043-898-0552 (all hours)
Office Phone No. for this study: 9347 4200

You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

2. PURPOSE OF THE STUDY

Approximately 4 years ago you joined this study and underwent clinical assessments at commencement (baseline) and at an 18 month and 36 month follow-up. The study has generated much valuable data on the genetic and lifestyle risk factors for Alzheimer's disease (AD) and biochemical profiles that may lead to an early diagnostic test for the condition.

From 2010, the study has approval to continue for a further 3 years. This will enable us to follow study participants over a longer period of time, increasing the quality and amount of valuable information to further develop risk profiles and diagnostic tests. In the extension, you will be invited back for 3 additional assessments at 18 month intervals (i.e. at 36, 54 and 72 months from commencement of the original study). The assessments will be similar to those you underwent at commencement and at 18 months, in that you will give a blood sample, perform memory tasks and complete questionnaires about your diet (one paper and pencil food frequency questionnaire (FFQ) i.e. CCV FFQ, and one computerised FFQ i.e. CSIRO FFQ) and exercise habits.

In addition, as part of the extension (i.e. 36, 54 and 72 month follow-ups), you will be asked to also provide a sample of your buccal (cheek) cells. The procedure for obtaining cheek cells is simply to wipe a disposable toothbrush over the cheek from inside your mouth. The collection will take under 5 minutes to conduct. We will analyse your buccal cells to identify a decrease in frequency of particular cells beyond the normal aging process that could detect Alzheimer's patients. This may reflect changes in the AD and Mild Cognitive Impairment (MCI) brain.

Memory loss is common among the general adult population. It may be nothing of clinical significance, a symptom of another condition such as depression, or an early sign of dementia such as the most common form of dementia, Alzheimer's disease (AD).

There is currently no method of clearly detecting whether a person is likely to develop AD or has AD, until the disease is well advanced. As treatment is likely to be most effective if given early, it is critical that researchers establish ways of diagnosing AD at an early stage.

This study aims to examine whether brain images, scores on psychometric tasks, neuropsychological (measurement of memory loss and thinking ability) tests, and/or blood biomarkers can help to diagnose AD at an early stage, or predict individuals who are at high risk for developing AD. This study will also examine lifestyle factors that may be related to AD, which may lead to the development of new treatments in the future.

This study is being carried out at research centres in Melbourne and Perth, with approximately 1000 participants in total. The following individuals over the age of 60 years will be eligible to participate in this study:

People with Alzheimer's disease (AD)

People with Mild Cognitive Impairment (MCI)

Healthy people, which can include carers of people with AD

This study has been reviewed and granted ethics approval by the Hollywood Private Hospital Research Ethics Committee and Edith Cowan University Human Research Ethics Committee, which oversee the ethical conduct of all research undertaken at the Sir James McCusker Alzheimer's Disease Research Laboratory.

3. PROCEDURES

We will perform the following tests and procedures at your 36, 54 and 72 month assessments:

Tests of Cognition (Thinking Ability), Memory Loss, Mood and Behaviour

You will be asked about your mood and behaviour, as this helps the study doctor understand if you may be at risk of depression. We will also test your memory and related thinking skills. These tests will take approximately one hour and 10 minutes to complete. The questions cover areas such as general knowledge, calculation, language, ability to recognise familiar objects and the ability to recall word lists.

Blood Sample

A nurse or other qualified person will take from you a blood sample using a sterile, disposable needle. The amount of blood to be taken will be 80 ml, which is equal to about 4 tablespoons. Researchers will then investigate the cells in your blood, along with the genetic information, known as DNA, contained in your genes.

We may also wish to use your blood and DNA in the future for related research and tests that are currently unknown or for other purposes. If this occurs then we have to obtain approval from the Hollywood Private Hospital and Edith Cowan University Human Research Ethics Committees.

Sometimes blood and/or DNA is sent to other research institutions within Australia and/or overseas. If this occurs, the researchers involved are required to demonstrate to a local Human Research Ethics Committee that the study meets the appropriate Australian ethical and privacy standards. If collaborative research is done with other research institutions, please be assured that your identity will not be disclosed to individuals working in these other institutions.

Your blood and DNA samples will be stored at the Sir James McCusker Alzheimer's Disease Research Laboratory, or at the premises of our research partners, until it is used up or until you contact us to request it be destroyed. Your samples will be labelled with a unique study code. This code protects your identity from technicians at the laboratory where the samples are analysed but allows your study doctor and members of the research team to identify your results. Procedures for secure and confidential storage of blood and DNA samples are those approved by the Hollywood Private Hospital and Edith Cowan University Human Research Ethics Committees.

Buccal (Mouth) Sample

You will need to provide a sample of your buccal (cheek) cells. The procedure for obtaining cheek cells is simply to wipe a disposable toothbrush over the cheek from inside your mouth. The collection will take under 5 minutes to conduct.

Consent for access to your Medical Records

In addition to asking your permission to utilise some of your blood for medical research, we also need to ask your permission for access to identified health information kept about you that is relevant to medical research. Such medical records may originate from hospitals, General Practice records, diagnoses by private specialists you have seen in the past, and information that is held on you by the Department of Health at the Health Information Centre.

Members of the research team may examine your health records. Any information obtained during the study is coded so that you cannot be identified. By signing the attached Consent Form, you authorise release of, or access to, this confidential coded information to the relevant study personnel as noted above. The review of these records may be in respect to this study and any further research that may be conducted in relation to it. These records will be made available, as described above, even if you withdraw.

OPTIONAL PROCEDURES

Physical Activity Monitoring – Actigraph Uni-axial Accelerometer

You may be invited to wear a small device called an actigraph to measure your physical activity levels. The actigraph is a small device (size of matchbox) that is not bulky, and is worn (at waist level) for the entire day excepting bathing and swimming. You will be asked to wear the actigraph for a period of 7 days, on three separate occasions. Each occasion will be up to 4 months apart. This will allow us to measure seasonal variation in your physical activity over a 12-month period. On each occasion, you will be required to come in 7 days later to return the actigraph, at which time a 'fasting' blood sample will be taken from you. The amount of blood to be taken on each occasion will be 80 ml, which is equal to about 4 tablespoons. We will measure levels of your blood constituents, such as lipids (cholesterol and triglyceride) and biomarkers related to AD and the results of these tests will be related to your activity level. If you would like some feedback on your blood test results and your activity level, please notify one of the researchers who will discuss these results with you. The Physical Activity Monitoring sub-study is NOT compulsory. You may continue to participate in the main study even if you decide not to participate in the Physical Activity Monitoring sub-study.

Body composition and bone density (DXA) scan

You may be invited to undergo a body composition and bone density (DXA) scan. This will take place on-site at Western Medicine, located within the Hollywood Private Hospital Specialist Centre, by trained professionals. This information will be used to investigate the association between body fat and Alzheimer's disease related proteins. The results of the scan will be discussed with you at the time of scanning with a qualified person from Western Medicine. You will also be given a copy

of these results and an information sheet identifying any lifestyle changes that can be made to improve your bone density. The procedure takes approximately 30-45 minutes and at no cost to you. As this is an optional part of the study you therefore do not have to participate in this component of the study.

Please be aware that the DXA scan may reveal you have a low bone mineral density and are at a high risk of bone fracture (osteoporosis). Should the scan reveal that you have osteoporosis, we will forward a specialist report to your GP. A copy of this report will be given to you. We will follow-up with you to ensure you have consulted your GP for further investigation and possible treatment for the condition. In addition, should you and your GP require assistance in managing osteoporosis arrangements can be made with a bone density specialist.

Neuroimaging

At each of the clinical assessments you will be invited to receive a magnetic resonance imaging (MRI) scan of your brain and either one or two positron emission tomography (PET) scans of your brain. You may also be invited to receive a PET scan of your pancreas. As these are optional parts of the study you therefore do not have to participate in these components. Please discuss any concerns that you may have regarding these imaging procedures with the research staff. For the PET and MRI scans, an additional visit for each scan will be required at each clinical assessment. If you agree to have your pancreas scanned this will take place on the same day as the PET scan of your brain.

PET Scans

The PET scans will be conducted at either the WA PET Cyclotron Services Department at Sir Charles Gairdner Hospital and/or the Oceanic Medical Imaging PET-CT Centre, Hollywood Private Hospital Medical Centre. You will be asked to fast for 6 hours prior to undergoing the scan. A small plastic needle will be inserted into a vein in your arm which will be used to inject a small dose of radioactive tracer (C11-PiB, F18-Flutemetamol, F18-Florbetaben, F18-Florbetapir, or F18-FDG) which will mix with your blood to travel to your brain and pancreas. You will then rest for 30 to 40 minutes, after which time you will lie in the PET scanner, holding your head and body as still as possible for about 30-40 minutes while pictures of your brain and pancreas where appropriate, are taken.

If you are invited to receive one PET scan of your brain, you will be injected with one of three new F18 radioactive tracers; either F18-Flutemetamol, F18-Florbetaben, or F18-Florbetapir. If you are invited to receive two PET scans of your brain you will be injected with C11-PiB and F18-FDG on separate occasions. The radiation dose from the injected tracers is relatively small, and is not considered to be a threat to your health. To put it into perspective, the radiation dose from the tracers is less than the dose you would receive from a conventional CT (X-ray) scan of the chest. Because your radiation exposure is low, you won't feel any effects and should be able to go home soon after your scan is complete. Most of the tracer will be flushed from your body within 6 to 8 hours. Other than the radiation exposure, there are no documented side effects associated with administration of PET tracers. In addition to the radiation risk, the insertion of the intravenous line

may cause bruising and very infrequently infection. Should a reaction or complication occur, you will be given appropriate treatment at the hospital.

If you are invited to receive an FDG-PET scan, on the day of your scan, upon arrival at the imaging centre, you will be asked to confirm that you have fasted and your blood glucose level will then be checked. Your blood glucose will be measured via a finger prick, in the same way that your pharmacist or GP may check your glucose levels. Your blood glucose level should be < 140 mg/dL (7.8 mmol/L). If your blood glucose level is 140 mg/dL or above, the FDG-PET scan may be re-scheduled. It is not necessary to check your blood glucose level on the day of any of the other PET scans, it is however, still essential that you fast for 6 hours prior to your scan.

MRI scans

The MRI scans will be conducted at SKG Radiology. It will require you to lie still in another scanner for 45 minutes. MRI is a non-invasive technique that enables images of internal parts of the human body to be generated without the use of ionising radiation such as X-rays. There are no injections involved in this procedure, nor will anything be removed from your body. However, MRI involves the use of a strong magnetic field to generate these images and as such, people with pacemakers, aneurysm clips, artificial heart valves, ear implants or metal/foreign objects in their eyes or elsewhere in their bodies face serious risk of injury. Thus if you think you may have any such implants or objects you should not participate in this part of the study. Furthermore, the MRI may cause anxiety and effects of claustrophobia in some people due to the loud noises generated by the machine and confined space in which the scan is performed.

Donation of a cerebrospinal fluid (CSF) sample

The brain and spinal cord are bathed in a clear fluid termed “cerebrospinal fluid” (CSF). CSF can be used to provide an insight into the functioning of the brain. This is in contrast to the blood, as the brain is protected by specialised blood vessels that prevent the passage of certain molecules into the brain. CSF is often sampled for diagnostic purposes, for example, the determination of bacterial meningitis, and is now becoming a substance of great interest in the Alzheimer’s field.

We would like to collect CSF (up to 20 ml, approximately 3 teaspoons of fluid) by a procedure termed a “lumbar puncture” (otherwise known as a “spinal tap”). The procedure involves the insertion of a fine needle between the vertebrae (bones that constitute the spine) in the lower back region and is performed under local anaesthesia and under strictly sterile conditions by an experienced and highly trained anaesthetist. As this is an optional part of the study you therefore do not have to participate in this component of the study. Please discuss any concerns that you may have regarding having a lumbar puncture procedure with the research staff.

As this is an invasive medical procedure, you will be required to attend the Hollywood Private Hospital Day Surgery Unit for a period of one to two hours, where the lumbar puncture will be performed and you will be monitored for any side effects (see Risks associated with participation in the optional sub-studies on pages 9, 10 and 11 of this information and consent form). As a further measure to ensure you do understand the nature and risks of a lumber puncture, we ask that you attend the day surgery clinic with a *trusted friend or relative. The anaesthetist will speak to the*

person accompanying you to confirm your capacity to fully understand what is involved in undergoing a lumbar puncture.

You will be asked to lie on your side in a curled-up position on a mattress. The skin will be cleaned with a disinfectant solution. A local anaesthetic will then be injected into the area to make it numb. A specialised needle will then be inserted between the vertebrae to collect the CSF. You will be asked to remain lying down for one hour while your condition is monitored. You will then be free to leave. The following day, you will receive a follow-up call from the anaesthetist to ensure that you are not experiencing any side effects.

We may wish to use the CSF that we collect from you in the future for related research and tests that are currently unknown or for other purposes. If this occurs then we have to obtain approval from the Hollywood Private Hospital and Edith Cowan University Human Research Ethics Committees.

Sometimes CSF is sent to other research institutions within Australia and/or overseas. If this occurs, the researchers involved are required to demonstrate to a local Human Research Ethics Committee that the study meets the appropriate Australian ethical and privacy standards. If collaborative research is done with other research institutions, please be assured that your identity will not be disclosed to individuals working in these other institutions.

Your CSF samples will be stored at the Sir James McCusker Alzheimer's Disease Research Laboratory, or at the premises of our research partners, until it is used up or until you contact us to request it be destroyed. Your samples will be labelled with a unique study code. This code protects your identity from technicians at the laboratory where the samples are analysed but allows your study doctor and members of the research team to identify your results. Procedures for secure and confidential storage of CSF samples are those approved by the Hollywood Private Hospital and Edith Cowan University Human Research Ethics Committees.

<u>Donation of a urine sample</u>

If you agree to donate a cerebrospinal fluid (CSF) sample, on the day that your CSF is collected, you will also be asked to provide a urine sample. If you agree to provide a urine sample, you will be given a collection cup and directed to a bathroom where you will collect 25 ml (approximately 4 teaspoons of fluid) of your own urine in private. Once collection is complete, you will return the collection cup to a nurse or researcher who will be located nearby. If you do not wish to donate a CSF sample you may still be asked to donate 25 ml of urine on the day that your blood sample is collected. As this is an optional part of the study you therefore do not have to participate in this component of the study.

<u>Cogstate Study</u>

You may be invited to participate in an additional study that will investigate the rate of change in cognitive function (i.e. thinking, reasoning and memory) in older adults. This study will commence from the time of your 36–month follow up visit. It will involve 10 sessions of up to 30 minutes each over the following 18 months.

You will be assessed with the Cogstate test, which is a computerised series of tasks which use playing cards. We can arrange for these sessions to take place at your home if you prefer. In addition, at four time points you will complete four brief neuropsychological measures, which will be familiar to you.

Subjective Memory Complaint Interview

If you are a healthy or Mild Cognitive Impairment participant, you may be asked to participate in an interview focusing on (a) your perception of your memory performance in hypothetical scenarios, and (b) details regarding your recent memories of personal events (for instance how you relive these memories and your reaction to situational demand on your memory). This study will also involve a short computerised task. This assessment will last approximately 55 minutes in total and will be audio recorded with your permission. In order to keep to a minimum the number of visits that you are asked to make to the research unit, if you agree to participate in this optional study component, a member of our research team will, with your consent, visit your home and conduct the interview there.

Study of Care-givers

If you are a participant with Alzheimer's disease or Mild Cognitive Impairment your next of kin or care-giver may be given the opportunity to complete a phone survey and paper questionnaires about care options and utilization of facilities and about the benefits of physical activity and how a physical activity program would be best implemented.

4. BENEFITS

You will not receive any direct benefit from participating in this study. If you are a patient with Alzheimer's disease or mild cognitive impairment, you may gain more information about your condition through education during the study. You will receive a report on your current nutritional intake, which will inform you of any excesses or deficiencies and allow you to adjust your diet if needed. As a research participant, you will also be contributing to the overall understanding and knowledge in the area of AD.

5. RISKS AND DISCOMFORTS

There are a number of potential risks associated with this study.

Risks associated with blood tests

There is a small risk of discomfort, bruising, and in extremely rare cases, infection at the site of the needle puncture, as a result of taking blood for laboratory testing. Some people feel dizzy or faint after they give blood. If you must come in for blood tests before a meal i.e. a fasted blood sample, you will be provided with an opportunity to eat before further testing is done. Food will be provided by the Study Group.

Risks associated with cognitive tasks

You may experience anxiety or psychological discomfort while completing the memory assessment.

Risks associated with providing personal information

As with the collection of any personal (private) information, there is a very slight risk of accidental disclosure of information or breach of computer security. Extensive safeguards are in place to minimize this potential risk, with hard copies of your information stored in locked cabinets within the principal researcher's office and electronic copies stored on file with password restricted access.

If you participate in the Body composition & bone density (DXA) scan sub-study

The DXA scan is a painless, non-invasive procedure and involves the use of X-rays to determine regional body fat composition and bone mineral density (bone strength). The radiation dose from one DXA scan is low, approximately $1/50^{th}$ of a chest X-ray. Your radiation exposure due to the DXA scan is the same amount you would receive during a 2 hour airplane flight and is less than normal background radiation you are exposed to on a yearly basis.

If you participate in the Actigraph Accelerometer sub-study

There is a very slight risk of increasing injury severity if the participant were to fall on the activity monitor but this is no greater than should they fall with anything else in their pocket or wearing a mobile phone or other tool on their belt.

If you participate in the optional MRI and PET brain imaging component:

Risks associated with MRI scans

The MRI scan does not cause any pain and does not expose you to X-ray radiation. However, MRIs use a magnetic field that can interact with medical devices or metal in your body. It is important that you inform the study team if you have any metal or metal devices or electrical parts in your body. The study doctor will go through a checklist with you. You do not need to tell the study team if you have fillings in your teeth, as they will have no effect. However, it is important that you tell your study team if you have tattoos, piercings, or permanent eye makeup. In addition, you will have to lie still on your back in the MRI scanner, which is a tight space. This may be difficult if you are claustrophobic. Some MRI machines are noisy and you may find this discomforting.

If you have previously had an MRI and experienced any unusual side effects, symptoms, or discomforts please tell the study team. If you experience any unusual side effects, symptoms, or discomforts during the MRI please tell the staff at your MRI appointment and tell the study team either at your next appointment or via a telephone call.

If you experience any disturbing symptoms at any time during the study or directly following your participation, you should contact the study doctor immediately.

Risks associated with PET scans

If you take part in the optional PET imaging, you will have a cannula (a small flexible tube with a needle end) inserted into a vein in your arm. The risks of having a cannula inserted are the same as those associated with blood tests, which are a small risk of discomfort, bruising, and on extremely rare occasions infection at the site of needle puncture.

This research study involves exposure to a small amount of radiation at each 18 month scan. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this research study will vary between 5.4 mSv and 7 mSv at each review, depending on whether you consent to undergo either one or two PET scans of your brain. If you consent to undergo one PET scan of your brain, you will be exposed to up to 7 mSv of radiation through the injection of one of three new F18 radioactive tracers; either F18-Flutemetamol, F18-Florbetaben, or F18-Florbetapir. If you consent to undergo two PET scans of your brain, you will be exposed to a total of 5.4 mSv of radiation through the injection of C11-PiB and F18-FDG on separate occasions. If you are also invited and consent to receive a PET scan of your pancreas, on the day of your C11-PiB brain scan you will additionally undergo a brief 'scouting' CT scan of your upper abdomen to locate the pancreas. This is accomplished easily with the combined CT-PET scanner but the 'scouting' CT scan entails extra radiation exposure of 0.5 mSv, thereby increasing the combined radiation dose to 5.9 mSv (C11-PiB and F18-FDG brain scans 5.4 mSv, + pancreas 0.5 mSv).

To put these radiation doses into perspective, 5.4 mSv is equivalent to two and a half years of natural background radiation that you would be exposed to through normal daily activities. 5.9 mSv and 7 mSv are equivalent to approximately three and three and a half years of natural background radiation respectively. This can be compared to, up to 10 mSv, which is the radiation dose you would be exposed to during a CT scan of the chest. Furthermore, the Australian Radiation Protection and Nuclear Safety Agency guidelines stipulate that it is acceptable for research participants over the age of 60 years to be exposed to up to 8 mSV per year in addition to radiation received as part of normal clinical management. The acceptable research dose increases to up to 12 mSV per year for research participants who are 70 years or more.

You should however, drink plenty of fluids afterwards to flush the radioactive drugs from your body. Most of the tracer will be flushed from your body within 6 to 24 hours. Other than the radiation exposure, there are no documented side effects associated with administration of PET tracers. It should be noted however, that C11-PiB, F18-Flutemetamol, F18-Florbetaben, and F18-Florbetapir are relatively new radiation markers and are currently only used for research purposes.

It is important to note that there are safety precautions for lactating women that must be adhered to. The F18-FDG tracer does not get into breast milk, but the person who receives the tracer emits a small amount of radiation for about 4 hours after the F18-FDG injection. Therefore, women should not have prolonged skin contact with a baby to breastfeed for at least 4 hours after the injection of the F18-FDG tracer. Breast milk can be safely pumped and fed to a baby by another person anytime after receiving the F18-FDG tracer.

<u>Risks associated with lumbar puncture</u>

The risks associated with lumbar puncture are greatly minimized when the procedure is performed by an experienced and highly trained medical professional under sterile conditions. You will also be monitored for a period of one hour in the Day Surgery unit to observe and treat any side effects, should they occur. In addition, you will receive a follow-up phone call the day after the procedure to ensure you are not suffering any side effects. However, there are some risks that you should carefully consider before agreeing to undergo a lumbar puncture. A lumbar puncture can

occasionally be uncomfortable or even painful, but the likelihood of this is minimized by the fact it is being performed by an experienced doctor and by the use of local anaesthetics. The most common side effect is headache, and, occasionally temporary sensations (such as tingling or numbness) can occur in the lower limbs. More rarely, bleeding and bruising at the lumbar puncture site and trauma to the spinal cord can occur, which can result in paralysis. Paralysis is however, extremely rare as the site of lumbar puncture is below that where nerves leave the spinal cord to enter the lower limbs. Infection can also occur, however this is reduced by using sterile instruments and cleaning the area with surgical disinfectants.

Additional possible risks

The possibility exists for a rare reaction to any of the procedures to which the participant will be exposed.

6. ALTERNATIVES

You do not have to participate in this study. Choosing not to participate will in no way affect your current or future medical care at any of the participating health services involved in this study. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage. This action will in no way affect your current or future medical care at any of the participating health services involved in this study.

7. PRIVACY, CONFIDENTIALITY AND DISCLOSURE OF INFORMATION

Any information obtained in connection with this research study that can identify you will remain confidential and will not be disclosed to any third party, except as required by law. The results of this research study may be presented at meetings or in publications. However, your identity will not be disclosed in those presentations. No patient will be identifiable by name in any publications/presentations arising from the study.

During the study, research information will be stored within the locked offices of Professor Ralph Martins at the Sir James McCusker Alzheimer's Disease Research Unit. The researchers named at the beginning of this consent form will have access to this information. Electronic data will be stored on computer with restricted access using password protection, with access limited to researchers within the study group.

It is desirable that your family GP be advised of your decision to participate in this research study. By signing the Consent Form, you agree to your family GP being notified of your decision to participate in this research study, and being kept informed of your progress in the study.

Other research centres may assist us with our research in exchange for our study findings and data. If this is the case, neither your name nor other information that can identify you personally will be given to these other research centres. Instead they will only be given data with an ID code, which only the researchers of this project at the Sir James McCusker Alzheimer's Disease Research Laboratory will be able to trace back to you. Therefore, please be assured that all personal information collected about you as part of this study will remain strictly confidential throughout the conduct of this study.

8. NEW INFORMATION ARISING DURING THE PROJECT

During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you will be told about this new information. This new information may mean that you can no longer participate in this research. If this occurs, the persons supervising the research will stop your participation. In all cases, you will be offered all available care to suit your needs and medical condition, if applicable.

9. RESULTS OF PROJECT

At the completion of the study, the results will be made available through either publication in a peer-reviewed journal, clinical meetings, and/or study reports. If you would like to be personally informed of the results, the researchers will provide you or your carer (if applicable) a verbal summary of the overall results of the study upon request. However, we wish to advise that information gained at early stages of any research will be preliminary in nature and is unlikely to have any relevance for you or your relative's health. You will not be informed of the results of the investigations other than if unexpected incidental pathology is detected such as a brain tumour or unknown stroke.

10. FURTHER INFORMATION OR ANY PROBLEMS

If you require further information or if you have any problems concerning this project you can contact the Principal researcher, Professor Ralph Martins, Ph: (08)9347 4201 or 0404-839-305; Associate researcher, Dr. Hamid Sohrabi, Ph: 0404-418-270; the Sir James McCusker Alzheimer's Disease Research Unit on (08)9347 4200; or Dr Mathew Samuel, ***Fremantle Hospital and the Fremantle Older Adult Mental Health Service (FOAMHS),*** Ph: (08)9347 4201.

If after agreeing to participate you have further questions or experience an adverse event, you are encouraged to contact us on the above numbers. Once having agreed to participate, you are free to withdraw from this study at any time without giving a reason and this will not affect your current or future medical care.

11. OTHER ISSUES

If you have any complaints about any aspect of the project, the way it is being conducted, or any questions about your rights as a research participant, then you may contact:

Dr. Terry Bayliss, Chairperson of the Hollywood Private Hospital Research Ethics Committee on (08)9346 6345, or Ms Kim Gifkins, Research Ethics Officer at Edith Cowan University on (08)6304 2170, or Secretary of the Sir Charles Gairdner Hospital Human Research Ethics Committee on (08)9346 2999, or Chair of the South Metropolitan Adult Health Service (SMAHS) Human Research Ethics Committee on (08)9431 2929, or Professor Frank Van Bockxmeer, Chairman of the Royal Perth Hospital Ethics Committee on (08) 9224 2244.

12. PARTICIPATION IS VOLUNTARY

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with the participating health services involved in this study.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. Please feel free to ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team of your intent.

13. ETHICAL GUIDELINES

This project will be carried out according to the *National Statement on Ethical Conduct in Research Involving Humans* (2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Hollywood Private Hospital, Edith Cowan University, Sir Charles Gairdner Hospital and South Metropolitan Adult Health Human Research Ethics Committees and the Ethics Committee of the Royal Perth Hospital.

The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

Participant Revocation of Consent Form

Version 16: 1st September 2012

I hereby wish to WITHDRAW my consent to participate in the research proposal named above and understand that such withdrawal WILL NOT jeopardize any treatment or my relationship with any of the participating Health Services.

Participant's Name (printed) _____

Signature: _____ Date_____

The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

PARTICIPANT CONSENT FORM Version 16: 1st September 2012
Consent for participation in an extension to the research project

I, _____of_____agree to continue my participation in an extension to "The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing". (Doctor or health professional) ……..…. has explained to me and I understand the consequences involved in my participation in the AIBL Flagship Study of Ageing. I have had an opportunity to ask questions and am satisfied with the answers given.
I understand my participation in the extension to this study will involve follow-up assessments at 36, 54 and 72 months, and that assessments will be similar to those I underwent at commencement and at 18 months.
I understand that participation in this project will involve:

1. Completion of questionnaires relating to my diet (one paper/pencil and one computerised) and exercise levels,
2. Measurement of mood, behaviour, cognition (thinking ability) and memory loss,
3. Voluntary Buccal (cheek) cell donation,
4. Voluntary donation of blood.

In making my donation, I freely agree to participate in this project according to the conditions in the Participant Information Form. I understand and agree that:
the blood (which in this consent form, includes its constituents, such as DNA, and any cell lines derived from the blood) and buccal cells will be stored and used in relation to The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing
the AIBL Flagship Study of Ageing will not be liable for any loss of, or damage to my blood, or derived cell constituents (including DNA) used in accordance with this form,
access to my blood or derived cell constituents (including DNA) for future unspecified research will only be released where the research proposal has been approved by a Human Research Ethics Committee,
access to my health information relevant to this study and any further research that may be conducted in relation to it, only where the research proposal has been approved by a Human Research Ethics Committee.
I also understand that:
I will be given a copy of the Participant Information and Consent Form to keep,
the researcher has agreed not to reveal my identity and personal details if information about this project is published or presented in any public form,
I am free to withdraw from this study at any time without giving a reason and without affecting my current or future medical care,
I will not benefit financially if this research leads to development of a new treatment or medical test,
I agree to my family GP being notified of my decision to participate in this research study, and being kept informed of my progress in the study.

<u>Optional parts of the research project; please CIRCLE your response:</u>

I give my consent to participate in the DXA scan sub-study	YES NO
I give my consent to participate in the Actigraph Accelerometer sub-study	YES NO
I give my consent to be contacted about PET and MRI imaging (including imaging of the pancreas)	YES NO
I may be contacted between visits to provide additional information or receive study updates	YES NO
I give my consent to be contacted about participation in future studies	YES NO
I give my consent to a lumbar puncture	YES NO
I give my consent to provide a urine sample	YES NO
I give my consent to participate in the Cogstate study	YES NO
I give my consent to participate in the Subjective Memory Complaint Interview	YES NO

Participant's Name (printed) _____
Signature_____Date_____

Declaration by researcher: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.
Researcher's Name (printed) _____
Signature_____Date_____

The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

PARTICIPANT CONSENT FORM Version 16: 1st September 2012

LUMBAR PUNCTURE: confirmation of research participant's capacity to give informed consent

This form is to be completed by the anaesthetist after consultation with the person accompanying the research participant to the lumbar puncture appointment.

I confirm, having assessed…….(research participant's name)

and having spoken to ……(name of person accompanying research participant)

of…….....(address of accompanying person),

who is the research participant's……..……(their relationship to research participant)

that the research participant demonstrates satisfactory understanding of the nature and risks of lumbar puncture procedure and a capacity to give informed consent.

Accompanying person's name (printed):_____

Signature:_____ Date:_____

Anaesthetist's name (printed):_____

Signature:_____Date:_____

Appendix 2

Validation study participant information and consent document

THE AUSTRALIAN IMAGING BIOMARKERS AND LIFESTYLE (AIBL) FLAGSHIP STUDY OF AGEING:

FOOD FREQUENCY QUESTIONNAIRE VALIDATION SUB-STUDY: INFORMATION AND CONSENT FORM

Version 2: 17th September 2013

Title of Study: The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing: Food Frequency Questionnaire Validation Sub-study

Principal Researcher: Professor Ralph N. Martins

Associate Researchers: Dr. Stephanie Rainey-Smith, Ms. Samantha Gardener.

This Participant Information and Consent Form is 8 pages long. Please make sure you have all the pages.

1. YOUR CONSENT

This Food Frequency Questionnaire Validation Sub-Study Information document contains detailed information about the research study. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this study before you decide whether or not to take part in it.

Please read this Food Frequency Questionnaire Validation Sub-study Information document carefully. Feel free to ask questions about any information in the document.

Once you understand what the study is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research study.

You will be given a copy of this document to keep as a record.

2. PURPOSE OF STUDY

The AIBL study in which you already participate, aims to improve diagnosis of Alzheimer's disease and hopes to discover treatments and lifestyle factors that can prevent or delay the disease. One of the preventative strategies that we are investigating is diet.

As part of the AIBL study you may have already completed a paper and pencil diet questionnaire which asked you to estimate how often and in what quantity you eat and drink certain foods and beverages. You may also have completed a computerised diet questionnaire which asked you to estimate how often and in what quantity you eat and drink a much longer list of foods and beverages.

We have modified the computerised diet questionnaire by adding foods and beverages that are of particular interest in the field of Alzheimer's research. These foods and beverages include items such as green tea, red wine, dark chocolate, pomegranates as well as any vitamin, mineral or dietary supplements you take.

Before we ask all AIBL study participants to complete the new version of the computerised diet questionnaire we first need to be sure of its ability to accurately and consistently estimate dietary intake of all the foods and beverages listed in the new version: this process is referred to as validation. If the new questionnaire is validated, the conclusions we make from the data collected using this questionnaire will be more reliable and will therefore be more valuable to the research community.

The process of validation involves completing a reliability and repeatability assessment, to ensure that people state the same answer to each question every time. Weighed food records are the best method for this validation as they are a record of actual intake of foods and beverages consumed, at the time of consumption.

If you agree to participate in this optional study we will compare the answers you give in the computerised diet questionnaire to a diary of the food and drink you consume over a four day period. You will be asked to complete the computerised diet questionnaire before the beginning and immediately after the end of the four day period and to keep a written record of everything you eat and drink during the nominated four days.

This study will be carried out at the McCusker Hollywood Private Hospital Alzheimer's Research Centre on the Hollywood Private Hospital campus in Nedlands, WA. Only individuals without a diagnosed memory problem will be eligible to participate.

3. PROCEDURES

Completing the Computerised Diet Questionnaire

You will be asked to complete the computerised diet questionnaire (CSIRO Food Frequency Questionnaire) before the beginning and immediately after the end of a four day period and to keep a written record (diary) of everything you eat and drink during the nominated four days.

The computerised questionnaire takes approximately 45-60 minutes to complete and can be completed over the internet at home, over the internet in our research centre or over the telephone. If you choose to answer the questionnaire over the telephone, a researcher will call you at a mutually convenient time and will ask you the questions over the telephone; the researcher will enter the answers you give into the computerised questionnaire whilst they are on the phone to you.

When completing the computerised questionnaire, the answers are automatically saved throughout so you can start and stop at times that are convenient, and you can complete the questionnaire over multiple sessions.

Completing the Written Food and Beverage Diary

You will be asked to keep a written record (diary) of everything you eat and drink for a period of four consecutive days that fall between the first and second time you complete the new version of the computerised diet questionnaire. This period of four consecutive days will start on a Sunday at midnight and finish on a Wednesday at midnight.

You will be asked to weigh the food you eat (after cooking, just before eating) and to record the weight on food diary sheets which will be provided to you. A set of scales will be provided to you, if possible we would like you to, weigh the food in grams using the scales provided. We will ask you to record both the portion served, and the left overs including bone skin, core, or that which is just not eaten. If weighing your food is not possible, we will ask you to approximate the amount of food as accurately as you can by size using measuring cups and spoons which we will provide to you. We will ask you to approximate the amount of beverage consumed as accurately as you can using measuring cups where no detailed measure of volume is available e.g. a 110 ml serving of red wine consumed in a restaurant.

You will be provided with detailed instructions (both written and oral) of how to complete your weighed food and beverage diary.

You will receive a phone call on the Monday to confirm there were no issues the preceding day and to answer any questions. You will also be provided with a mobile telephone number which can be called anytime to discuss questions or problems that may arise over the four days.

4. STUDY SCHEDULE

If you agree to participate in this study you will be asked to attend the research centre on two occasions (this number increases to four occasions if you decide to complete the computerised diet questionnaire at the research centre).

Participating in this study involves the following 5 steps:

1. Completion of a computerised diet questionnaire (this will take between 45 and 60 minutes and needs to be completed over the internet; your answers are automatically saved so you can complete it over multiple sessions).
2. Attend a meeting at the research centre to receive instructions (written and oral) on how to complete a 4 day weighed food diary (diary sheets, a set of scales and measuring cups and spoons will be provided) (60-90 minutes). There may be other individuals who are participating in this study present at this meeting; please advise a researcher if you would prefer to meet with the researcher alone.
3. Completion of a 4 day weighed food record (Sunday to Wednesday); this involves weighing and recording all food and drink you consume in these 4 days.
4. Final meeting to return equipment, discuss any issues and go through your food diary (30-60 minutes); no other participants will be present.
5. Completion of the same online diet questionnaire (45-60 minutes).

5. BENEFITS

You will not receive any direct benefit from participating in this study. You will receive a report on your current nutritional intake each time you complete the CSIRO FFQ, which will inform you of any excesses or deficiencies and allow you to adjust your diet if needed. As a research participant, you will also be contributing to the overall understanding and knowledge in the area of Alzheimer's disease.

6. RISKS AND DISCOMFORTS

There are no foreseeable risks to you.

7. ALTERNATIVES

You do not have to participate in this study; participation is voluntary. Choosing not to participate will in no way affect your current or future medical care at any of the participating hospitals involved in this research study; including Hollywood Private Hospital.

8. PRIVACY, CONFIDENTIALITY AND DISCLOSURE OF INFORMATION

Any information obtained in connection with this research study that can identify you will remain confidential and will only be used for the purpose of this study. It will only be disclosed with your permission, except as required by law. The results of this research study may be presented at research meetings or in peer-reviewed publications; however, your identity will not be disclosed in those presentations. No participant will be identifiable by name in any publications/presentations arising from the study. Each participant in this study is given a unique study identification code to identify all data related to that participant. This code is the only means of identification which is used in any analysis, presentation of results, or communication of results with any other researchers outside of the team for this study.

During the study, research information will be stored within locked offices at the McCusker Hollywood Private Hospital Alzheimer's Research Centre. The researchers named at the beginning of this document are the only people who will have access to this information. Electronic data will be stored on computer with restricted access using password protection, with access limited to researchers within the study group.

9. RESULTS OF PROJECT

At the completion of the study, the results will be made available through either publication in a peer-reviewed journal, clinical meetings, and/or study reports. If you would like to be personally informed of the results, the investigators will provide you with a verbal summary of the overall results of the study upon request.

10. FURTHER INFORMATION OR ANY PROBLEMS

If you require further information or if you have any problems concerning this project you can contact the Principal researcher, Professor Ralph Martins, Ph: (08) 9347 4200 or 0404-839-305; Associate researcher, Dr. Stephanie Rainey-Smith, Ph: 043-898-0552; or the McCusker Alzheimer's Research Foundation on (08) 9347 4200.

11. OTHER ISSUES

If you have any complaints about any aspect of the project, the way it is being conducted, or any questions about your rights as a research participant, then you may contact any of the people below who represent the human ethics research committees of the research institutions involved in this study: Dr. Terry Bayliss, Chairperson of the Hollywood Private Hospital Research Ethics Committee (08) 9346 6345, Ms Kim Gifkins, Research Ethics Officer at Edith Cowan University (08) 6304 2170.

12. PARTICIPATION IS VOLUNTARY

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to do so. Furthermore, if you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with any one of the participating Health Services.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. You can ask for any information you wish. If you agree to participate in this study, please sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, we ask that you please notify a member of the research team before you withdraw.

13. ETHICAL GUIDELINES

This project will be carried out according to the *National Statement on Ethical Conduct in Research Involving Humans* (June 1999) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Hollywood Private Hospital and Edith Cowan University.

The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

FOOD FREQUENCY QUESTIONNAIRE VALIDATION SUB-STUDY CONSENT FORM

Version 2: 17th September 2013

- I have had an opportunity to ask questions and I am satisfied with the answers I have received.
- I freely agree to participate in this project according to the conditions outlined in the Food Frequency Questionnaire Validation Sub-study Information document.
- I understand that I am free to withdraw from this sub-study at any time without giving a reason and without affecting my current or future medical care nor my ongoing involvement in the main AIBL study
- I will be given a copy of the Food Frequency Questionnaire Validation Sub-study Information and Consent Form to keep.
- I will complete the online CSIRO Food Frequency Questionnaire before and after I complete my weighed food record.
- Where possible, I will weigh or measure all food and beverages consumed during a specified four day period, and will record this information in the food diary.
- I understand that the researcher has agreed not to reveal my identity and personal details if information about this project is published or presented in any public form.
- I will provide information to the best of my knowledge and ability.

Participant's Name (printed)

_____

Signature_____Date_____

Declaration by researcher: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher's Name (printed)

_____

Signature_____Date_____

Note: All parties signing the Consent Form must date their own signature.

The Australian Imaging Biomarkers and Lifestyle (AIBL) Flagship Study of Ageing

FOOD FREQUENCY QUESTIONNAIRE VALIDATION SUB-STUDY

REVOCATION OF CONSENT FORM

Version 2: 17th September 2013

I hereby wish to WITHDRAW my consent to participate in the research study named above and understand that such withdrawal WILL NOT jeopardize any treatment or my relationship with any of the participating Health Services.

Participant's Name (printed)

_____

Signature_____Date_____

Appendix 3

Validation study four day weighed food diary

WEIGHED FOOD RECORDS STUDY – 4 DAY FOOD DIARY

Time of day	Food	Description of food	Amount	Weight	Waste	Where the food was eaten	Cooking method
EXAMPLE: 12.30pm	Salad Sandwich	Cos lettuce Wholemeal bread (Tip Top) Margarine (Nuttelex) Hi Low Milk (Brownes)	2 leaves 2 slices 1 teaspoon 1 cup	14g 64g 5g 220ml	Crust 5g	At home	None

Name.....Day of Week.....

Appendix 4

Pearson's and intra-class correlation coefficients for the reliability of the modified Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire

Food item from modified CSIROFFQ	Food section	Pearson's correlation coefficient	*p*-value	Intra-class correlation coefficient	*p*-value
Cereals	Cereals	0.665	***	0.665	***
Bread rolls	Cereals	0.142		0.106	
Wheat germ	Cereals	0.974	***	0.974	***
Linseed, sunflower seed and almond mixture	Cereals	0.693	***	0.676	***
Fried rice	Cereals	0.421	**	0.404	**
Risotto	Cereals	0.577	***	0.529	***
Packet pasta and sauce	Cereals	0.197		0.174	
Instant noodles	Cereals	0.380	**	0.356	**
Plain pasta	Cereals	0.837	***	0.836	***
Filled pasta	Cereals	0.717	***	0.708	***
Asian noodles	Cereals	0.887	***	0.770	***
Flat breads	Cereals	0.960	***	0.945	***
Crumpets or English muffins	Cereals	0.957	***	0.956	***
Croissants	Cereals	0.978	***	0.433	***
Fruit loaf or currant bread	Cereals	0.671	***	0.665	***
Crisp bread, rice cake or cracker	Cereals	0.585	***	0.550	***
Salted biscuits	Sweets and snacks	0.576	***	0.552	***
Plain sweet biscuits	Sweets and snacks	0.547	***	0.422	***
Fancy sweet biscuits	Sweets and snacks	0.370	**	0.352	**
Muesli bar or health bar	Sweets and snacks	0.783	***	0.781	***
Cake or sweet muffin	Sweets and snacks	0.788	***	0.763	***
Fruit pie, pastry or fritters	Sweets and snacks	0.386	**	0.382	**

Cereal-based sweet desserts	Sweets and snacks	0.667	***	0.450	***
Sweet bun or doughnut	Sweets and snacks	0.831	***	0.830	***
Potato crisps, twisties or corn chips	Sweets and snacks	0.303	*	0.248	*
Popcorn	Sweets and snacks	0.444	***	0.366	**
Chocolate covered bar	Sweets and snacks	0.954	***	0.953	***
Lollies or toffees	Sweets and snacks	0.437	**	0.432	***
Pate or liver paste	Sweets and snacks	0.935	***	0.902	***
Milo, Quik, Ovaltine, cocoa or hot chocolate	Beverages	0.577	***	0.560	***
Flavoured milk	Beverages	0.967	***	0.293	*
Milkshake or thick shake	Beverages	0.905	***	0.905	***
Glass of milk	Beverages	0.577	***	0.444	***
Coffee	Beverages	0.447	***	0.446	***
Sugar or honey added to hot beverages	Beverages	0.942	***	0.942	***
Herbal tea	Beverages	0.599	***	0.421	***
Cheese	Dairy	0.802	***	0.801	***
Cottage cheese	Dairy	0.744	***	0.593	***
Soft cheese	Dairy	0.249		0.201	
Ricotta cheese	Dairy	0.489	***	0.476	***
Cream	Dairy	0.433	**	0.305	*
Dairy style dessert	Dairy	0.680	***	0.211	
Yoghurt	Dairy	0.459	***	0.454	***
Ice cream	Dairy	0.922	***	0.909	***
Ice block or icy pole	Dairy	0.323	*	0.302	*
Custard	Dairy	0.980	***	0.980	***
Fried egg	Dairy	0.686	***	0.686	***

Boiled or poached egg	Dairy	0.798	***	0.791	***
Omelette or scrambled eggs	Dairy	0.577	***	0.570	***
Steak	Meat	0.873	***	0.846	***
Pork chop	Meat	0.743	***	0.710	***
Lamb chop or cutlet	Meat	0.793	***	0.774	***
Roast pork or pork fillet	Meat	0.524	***	0.524	***
Roast beef or veal	Meat	0.570	***	0.570	***
Roast lamb	Meat	0.497	***	0.454	***
Crumbed veal	Meat	0.858	***	0.847	***
Sausages	Meat	0.822	***	0.772	***
Rissole or meat patty	Meat	0.437	**	0.413	***
Frankfurters or saveloys	Meat	1.000	***	0.473	***
Bacon	Meat	0.839	***	0.813	***
Ham	Meat	0.665	***	0.641	***
Luncheon meat	Meat	0.822	***	0.234	
Continental sausage	Meat	0.782	***	0.769	***
Vegetarian curry, stew or casserole	Meat	0.638	***	0.635	***
Asian stir-fry	Meat	0.622	***	0.602	***
Vegetarian stir-fry	Meat	0.490	***	0.484	***
Mornay dishes	Meat	0.664	***	0.645	***
Mincemeat dish	Meat	0.428	**	0.432	***
Mincemeat as a sauce	Meat	0.644	***	0.567	***
Pizza	Meat	0.498	***	0.496	***
Meat pie	Meat	0.366	**	0.357	**
Savoury pies / pastries	Meat	0.331	**	0.191	
Canned fish	Fish	0.587	***	0.573	***

Roast / barbeque chicken	Poultry	0.699	***	0.679	***
Chicken breast/thighs/wings	Poultry	0.455	***	0.430	***
Chicken cooked in simmer sauce	Poultry	0.587	***	0.250	*
Crumbed chicken	Poultry	0.520	***	0.510	***
Steamed, grilled or boiled fish	Fish	0.785	***	0.753	***
Fried fish	Fish	0.521	***	0.494	***
Oven baked fish	Fish	0.524	***	0.517	***
Fish fingers	Fish	0.336	*	0.254	*
Seafood	Fish	0.219		0.195	
Pizza	Takeaway foods	0.491	***	0.399	**
Chicken or fish burgers	Takeaway foods	-0.040		-0.017	
Hamburgers	Takeaway foods	0.524	***	0.505	***
Souvlaki or gyro	Takeaway foods	-0.026		-0.003	
Subs or wraps	Takeaway foods	0.039		0.035	
Chicken nuggets	Takeaway foods	-0.021		-0.015	
Deep fried chicken	Takeaway foods	0.241		0.101	
Deep fried battered fish	Takeaway foods	0.739	***	0.738	***
Deep fried seafood	Takeaway foods	0.516	***	0.507	***
Potato cakes or fritters	Takeaway foods	0.420	**	0.404	**
Spring or Chiko roll	Takeaway foods	0.425	**	0.326	**
Fried dim sim	Takeaway foods	0.284	*	0.258	*
Steamed dim sim	Takeaway foods	0.361	*	0.180	
Meat or chicken pie	Takeaway foods	0.474	***	0.429	***
Pastie	Takeaway foods	0.906	***	0.821	***
Sausage roll	Takeaway foods	0.671	***	0.671	***

Sushi	Takeaway foods	0.793	***	0.723	***
Fries	Takeaway foods	0.658	***	0.620	***
Hot chips	Takeaway foods	0.237		0.150	
Garlic bread	Takeaway foods	0.512	***	0.467	***
Baked beans	Vegetables	0.618	***	0.609	***
Other beans - dried or canned	Vegetables	0.873	***	0.819	***
Lentils - dried or canned	Vegetables	0.759	***	0.577	***
Sweetcorn - canned	Vegetables	0.653	***	0.652	***
Beetroot - canned	Vegetables	0.886	***	0.876	***
Olives	Vegetables	0.795	***	0.780	***
Gherkins or pickled onions	Vegetables	0.861	***	0.837	***
Other pickled vegetables	Vegetables	0.942	***	0.784	***
Thick sauces	Miscellaneous	0.614	***	0.578	***
Gravy	Miscellaneous	0.371	**	0.360	**
Mayonnaise	Miscellaneous	0.702	***	0.680	***
Salad dressing	Miscellaneous	0.864	***	0.864	***
Marinades and other thick sauces	Miscellaneous	0.768	***	0.767	***
Potato - mashed	Vegetables	0.924	***	0.923	***
Potato - boiled	Vegetables	0.052		0.043	
Potato - roasted	Vegetables	0.594	***	0.594	***
Onion - fried	Vegetables	0.330	**	0.328	**
Onion - raw, baked or boiled	Vegetables	0.442	***	0.435	***
Tomato	Vegetables	0.402	**	0.402	**
Mushrooms - fresh	Vegetables	0.524	***	0.459	***
Mushrooms - fried	Vegetables	0.105		0.082	

Carrots	Vegetables	0.590	***	0.584	***
Turnip or swede	Vegetables	0.451	***	0.320	*
Green beans	Vegetables	0.578	***	0.546	***
Green peas	Vegetables	0.428	**	0.391	**
Zucchini	Vegetables	0.862	***	0.847	***
Cabbage	Vegetables	0.871	***	0.850	***
Brussels sprouts	Vegetables	0.889	***	0.875	***
Silverbeet or spinach	Vegetables	0.416	**	0.415	***
Broccoli	Vegetables	0.488	***	0.488	***
Cauliflower	Vegetables	0.434	**	0.366	**
Pumpkin	Vegetables	0.591	***	0.577	***
Sweetcorn	Vegetables	0.547	***	0.475	***
Lettuce	Vegetables	0.194		0.186	
Cucumber	Vegetables	0.671	***	0.656	***
Celery	Vegetables	0.465	***	0.458	***
Bean sprouts	Vegetables	0.197		0.140	
Capsicum	Vegetables	0.276		0.228	
Fried mixed vegetables (e.g. stir fry) not accounted for elsewhere	Vegetables	0.384	**	0.362	**
Oven baked hot chips prepared at home	Vegetables	0.909	***	0.909	***
Coleslaw	Miscellaneous	0.668	***	0.664	***
Potato salad	Miscellaneous	0.790	***	0.748	***
Orange, mandarin or grapefruit	Fruit	0.502	***	0.433	***
Apple or pear	Fruit	0.577	***	0.565	***
Banana	Fruit	0.201		0.148	
Pineapple	Fruit	0.695	***	0.685	***
Avocado	Fruit	0.673	***	0.669	***

Fresh fruit salad	Fruit	0.460	***	0.382	**
Melon	Fruit	0.402	**	0.395	**
Watermelon	Fruit	0.526	***	0.490	***
Peach	Fruit	0.477	***	0.354	**
Plum	Fruit	0.783	***	0.783	***
Nectarine	Fruit	0.208		0.199	
Apricot	Fruit	0.582	***	0.582	***
Figs	Fruit	0.799	***	0.760	***
Canned fruit	Fruit	0.956	***	0.912	***
Raisins sultanas or currants	Fruit	0.490	***	0.490	***
Other dried fruit	Fruit	0.549	***	0.421	***
Honey, jam or marmalade	Miscellaneous	0.791	***	0.788	***
Vegemite or marmite	Miscellaneous	0.779	***	0.747	***
Peanut paste	Miscellaneous	0.513	***	0.433	***
Cordial	Beverages	0.852	***	0.851	***
Diet fizzy drink	Beverages	0.694	***	0.661	***
Regular fizzy drink including cola drinks and flavoured mineral water	Beverages	0.445	***	0.445	***
Fruit drink	Beverages	0.953	***	0.952	***
Pure fruit juice	Beverages	0.772	***	0.746	***
Vegetable juice	Beverages	0.653	***	0.426	***
Water or spring water	Beverages	-0.181		-0.174	
Unflavoured mineral water	Beverages	0.437	**	0.435	***
Rum and soda	Beverages	1.000	***	1.000	***
Low-alcohol beer	Beverages	0.908	***	0.906	***
Regular beer	Beverages	0.889	***	0.862	***

Sherry, port or liqueur	Beverages	0.708	***	0.570	***
Alcoholic spirits	Beverages	0.979	***	0.946	***
Homemade soup	Miscellaneous	0.803	***	0.801	***
Canned soup	Miscellaneous	0.706	***	0.705	***
Packet soup	Miscellaneous	0.817	***	0.796	***
Barley	Vegetables	0.913	***	0.913	***
Bok Choy	Vegetables	0.475	***	0.465	***
Couscous	Vegetables	0.626	***	0.497	***
Garlic	Vegetables	0.507	***	0.478	***
Pomegranates	Fruit	0.951	***	0.726	***
Sweet potato	Vegetables	0.405	**	0.404	**
Canned tomatoes	Vegetables	0.230		0.224	
Cherries	Fruit	0.278		0.274	*
Kiwifruit	Fruit	0.981	***	0.949	***
Mango	Fruit	0.693	***	0.612	***
Pawpaw	Fruit	0.916	***	0.592	***
Milk	Dairy	0.270		0.269	*
Chocolate	Miscellaneous	0.441	***	0.389	**
Wine and champagne	Beverages	0.873	***	0.873	***
Black tea	Beverages	0.415	**	0.391	**
Green tea	Beverages	0.427	**	0.400	**
White tea	Beverages	0.751	***	0.751	***
Pomegranate juice	Beverages	0.760	***	0.557	***
Red or black grapes	Fruit	0.047		0.033	
Green grapes	Fruit	0.453	***	0.453	***
Seeds	Miscellaneous	0.800	***	0.794	***
Berries	Fruit	0.293	*	0.167	
Liver and kidney	Meat	0.628	***	0.362	**

Meat goulash, curry, stew or casserole	Meat	0.766	***	0.763	***
Rice	Cereals	0.722	***	0.713	***
Nuts	Miscellaneous	0.708	***	0.650	***
Jacket potatoes	Vegetables	0.890	***	0.856	***
Fats and oils	General	0.308	*	0.306	*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: CSIROFFQ, Commonwealth Scientific and Industrial Research Organisation food frequency questionnaire.