Preventive potential of diet in the pre-clinical phase of Alzheimer's disease symptomatology

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ABSTRACT

Projections estimate 131.5 million will be living with dementia by the year 2050. Alzheimer's disease is the leading cause of dementia, accounting for 60-70% of all cases and is a global public health priority. Alzheimer's disease (AD) risk increases with age and lacks efficacious drugs. Pharmacological treatment is failing, leading to a growing body of research investigating the preventative potential of modifiable lifestyle risk factors, such as diet. Summaries of the existing evidence reveal an association between Mediterranean-style diet adherence and reduced AD incidence; however, no review has investigated this relationship with respect to the hallmark AD biomarkers that manifest decades prior to clinical symptomatology. Amassed evidence indicates associations between diet and Alzheimer's disease may occur through biomarker pathways such as amyloid-β; however, very few studies have investigated dietary/ amyloid-β relationships and prior to this thesis, no study has investigated this relationship in a female only cohort.

Chapters 1 and 2 examined the current literature regarding diet-AD relationship. Previous evidenc from systematic review suggests a relationship between dietary adherence and Alzheimer's risk; however, this thesis provided the first meta-analytic evidence of this relationship extending to the prodromal phase of neuropathological change. A comprehensive systematic review and meta analysis into diet and Alzheimer's biomarkers found a small but significant effect of diet on AD biomarkers. This review supported the notion that diet and nutrition display therapeutic potential for non-pharmacological lifestyle intervention.

Chapters 4 and 5 investigated the changing nutritional and dietary habits of Australian ageing women over time. Participants from the longitudinal Women's Healthy Ageing Project completed assessments, including a validated food frequency questionnaire, at two time-points 14 years apart (1998 and 2012). Energy intake significantly decreased over time, whilst energy-adjusted total fat, saturated fat, monounsaturated fat and cholesterol intakes all significantly increased. Three dietary patterns were identified at both time points; two 'healthy-type' patterns as well as a third less healthy pattern. In these women, although some participant's dietary pattern remained largely stable over time, the majority of women underwent dietary pattern change over this time in their lives.

Chapter 6 presented a cross-sectional analysis of the relationship between dietary pattern adherence and beta-amyloid deposition in participants of the Women's Healthy Ageing Project. Adherence to the Junk Food dietary pattern was found to be a significant predictor of cerebral amyloid-β deposition, highlighting the importance of diet as a potentially modifiable lifestyle risk factor in the preclinical phase of Alzheimer's disease.

This thesis presented the first systematic review and meta analysis analysing diet-Alzheimer's relations in the preclinical, neuropathological stage of disease progression. Given accumulated evidence of a diet-Alzheimer's pathway in the hallmark biomarkers of this disease, this thesis was the first to examine this relationship in a female only cohort, at a much greater risk than their male counterparts. The results of this thesis, in concert with previous literature, suggest diet has a substantial role in preclinical Alzheimer's disease progression and displays significant therapeutic potential as a low-cost, accessible and effective modifiable lifestyle risk factor. However; it is clear that the complex connection between diet and

brain health requires further research to elucidate the underlying mechansisms governing this relationship.

DECLARATION

This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No parts of this thesis have been submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgment in the main text of the thesis.

All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required).

In pursuit of this research and the preparation of this thesis, collaborations were completed with Prof Cassandra Szoeke, Prof Peter Clifton, Dr Allison Hodge, Dr Nitin Shivappa, Dr James Hebert, Prof Lorraine Dennerstein AO, Dr Alicia Goodwill, Alexandra Gorelik and Dr Xianwen Shang. Statistical analysis assistance was received from Dr Alicia Goodwill, Alexandra Gorelik and Dr Steven Simpson Jr.

Signed: 

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“In examining disease, we gain wisdom about anatomy and physiology and biology. In examining the person with disease, we gain wisdom about life.”

Oliver Sacks, The Man Who Mistook His Wife for a Hat, 1985

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LIST OF ABBREVIATIONS AND ACRONYMS

^{11}C = N-methyl-[11C]2-(4=-methylaminophenyl)-6-

(^{18}F) AV-45 = Florbetapir (E)-4-(2-(6-(2-(2-(2-([18F]-

(^{18}F) BAY94–9172 = Florbetaben

(^{18}F) GE067 = Flutemetamol

AA = Alzheimer's Australia

Aβ = Beta-Amyloid

ADAS-Cog = Alzheimer Disease Assessment Scale

ADNI = Alzheimer's Disease Neuroimaging Initiative

aMCI = Amnestic Mild Cognitive Impairment

ANOVA = Analysis of Variance

ANCOVA = Analysis of Covariance

APOE-ε4 = Apolipoprotein E epsilon 4 allele

APP = Amyloid Precursor Protein

AIHW = Australian Institute of Health and Welfare

AUD = Australian Dollars

BBB = Blood Brain Barrier

BDNF = Brain-Derived Neurotrophic Factor

BMI = Body Mass Index

BMD = Bone Mineral Density

CAE = Centre for Adult Education

CI = Confidence Interval

CNS = Central Nervous System

CRP = C-Reactive Protein

CSF = Cerebrospinal Fluid

DES = Discrete Event Simulation

DEXA = Dual-Energy X-ray Absorptiometry

DHQ = Diet History Questionnaire

DII = Dietary Inflammatory Index

DSM = Diagnostic and Statistical Manual of Mental Disorders

ELISA = Enzyme-Linked Immunosorbent Assay

FDDNP = 2-(1-6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl-ethylidene)

FDG = 18F-2-fluoro-2-deoxy-D-glucose

FFQ = Food Frequency Questionnaire

GBD = Global Burden of Disease

HGLD = High Glycemic Load Diet

HREC = Human Research Ethics Committee Study

ICD = International Classification of Disease

IL = Interleukin

LT = Longitudinal

MAP = Microtubule Associated Protein

MD/MeDi = Mediterranean Diet

MET = Metabolic Equivalent of Task

MMSE = Mini Mental Status Examination

MSE = Modified Mini-Mental Status Examination

MUFA = Monounsaturated Fatty Acid

MWMHWP = Melbourne Women's Midlife Health Project

NIA-AA = National Institute on Aging-Alzheimer's Association

NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association

NFT = Neurofibrillary Tangles

NL = Cognitively Normal

NL-ENIGMA = Effect of a specific Nutritional Intervention on cerebral Glucose Metabolism in early Alzheimer's disease

NPI = Neuropsychiatric Inventory

NPE = Neuropsychological Evaluation

OR = Odds Ratio

PA = Physical Activity

PCA = Principal Component Analysis

PCC = Posterior Cingulate Cortex

PET = Positron Emission Tomography

PiB = Pittsburgh Compound-B

PiB-BP = Pittsburgh Compound-B Binding Potential

PLS = Partial Least Squares

P-tau = Phosphorylated Tau

PUFA = Polyunsaturated Fatty Acid

RCPM = Raven's Colored Progressive Matrices

RCT = Randomised Control Trial

RNA = Ribonucleic Acid

SE = Standard Error

SFA = Saturated Fatty Acid

siRNA = Small Interfering Ribonucleic Acid

SUVR = Standardised Uptake Value Ratio

T-tau = Total Tau

TAFE, Technical and Further Education

TNF-α - Tumor Necrosis Factor Alpha

USD = United States of America Dollars

WHO = World Health Organization

XS = Cross Sectional

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1 INTRODUCTION

This chapter describes the background and contextual framework for this thesis, including the epidemiological evidence for Alzheimer's disease (AD), AD's neuropathological features and both modifiable and non-modifiable risk factors for disease progression. AD will be discussed in an epidemiological, social and physiological framework. Evidence for the incidence and prevalence of worldwide AD will be collated and described. The impact of AD on the social spheres will be elaborated upon, as well as the clinical and neuropathological features for this disease. Methods of diagnosing AD will be discussed in depth and finally the evidence of risk factors, both modifiable and non-modifiable, for AD will be analysed in detail through both a clinical and pathological perspective.

1.1 The Alzheimer's disease epidemic

AD is the most common form of dementia and is a global health epidemic, with 40-50 million people currently living with dementia worldwide [1-3]. In 2012 there were approximately 35.6 million people with AD worldwide and estimates indicate that this number will increase to 115.4 million by 2050[1]. The global population is ageing and research has projected that by 2050 the number of elderly people (aged 65 years or older) will double the number of children (aged 0-14 years) for the first time in history[4]. In the absence of a cure, the impetus to examine risk factors is clear [5].

1.1.1 Incidence and prevalence of Alzheimer's disease

Globally the number of deaths due to dementia between 1990 and 2016 increased by 148% [5]. The number of prevalent dementia cases increased by 117% from 20.2 million (1990) to 43.8 million (2016)[5]. In the 195 countries included in the 2016 Global Burden of Disease (GBD) study, it was estimated that in 2016, 27.0 million women and 16.8 million men were living with dementia[5] (Figure 1). Dementia is the fifth leading cause of death globally[5] and the leading cause of death in women in Australia[6].

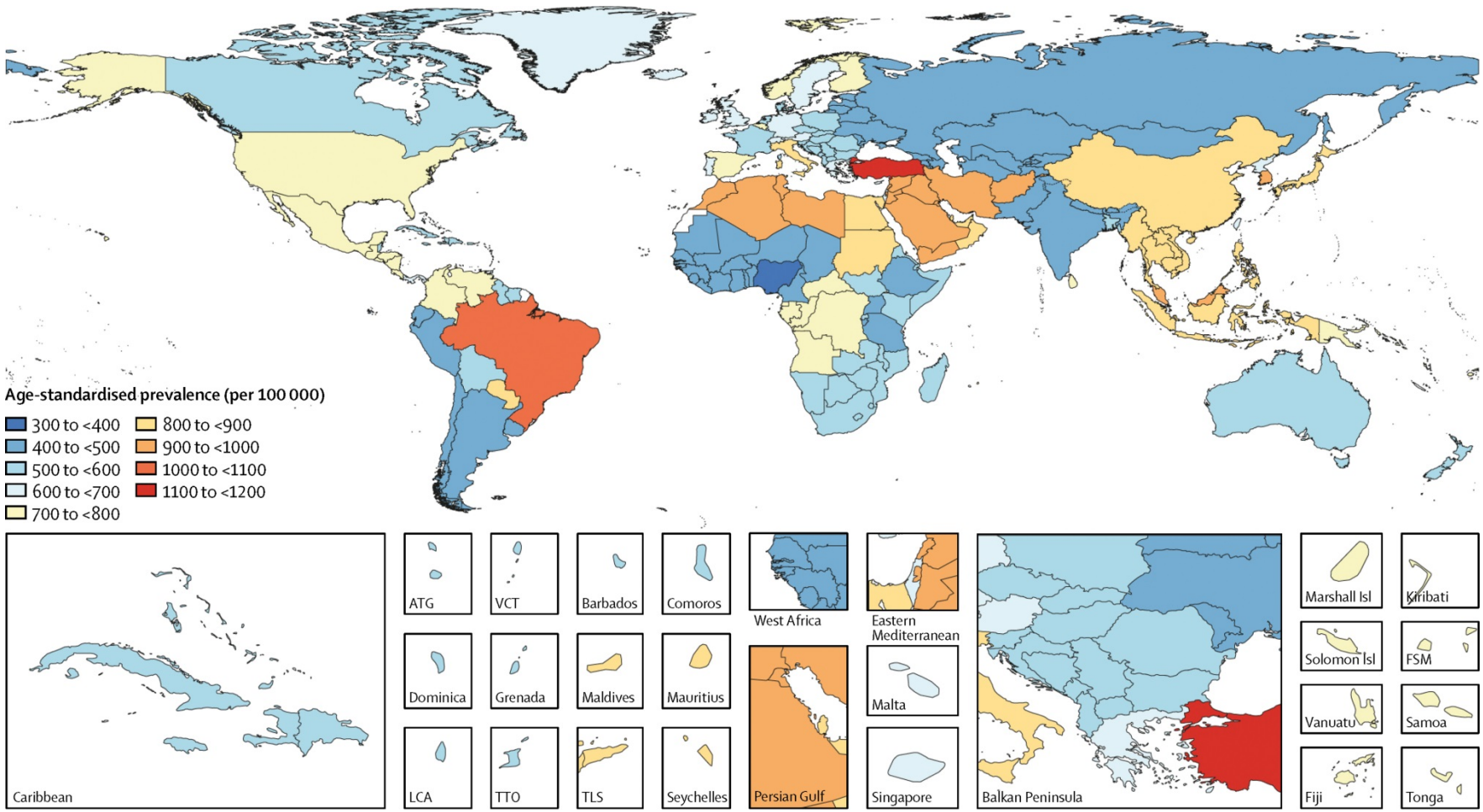

Figure 1: Age-standardised prevalence for Alzheimer's disease and other dementias per 100 000 population by location for both sexes, 2016[5]

Simulation modelling predicts the prevalence of dementia in Australia is set to rise to 387,000 in 2020 and 928,000 on 2050[7] (Figure 2). Standfield, *et al.* [7] compared a novel individual patient discrete event simulation (DES) modelling technique with estimates from the Australian Institute of Health and Welfare (AIHW)[8], Deloitte Access Economics[9] and Anstey, *et al.* [10]

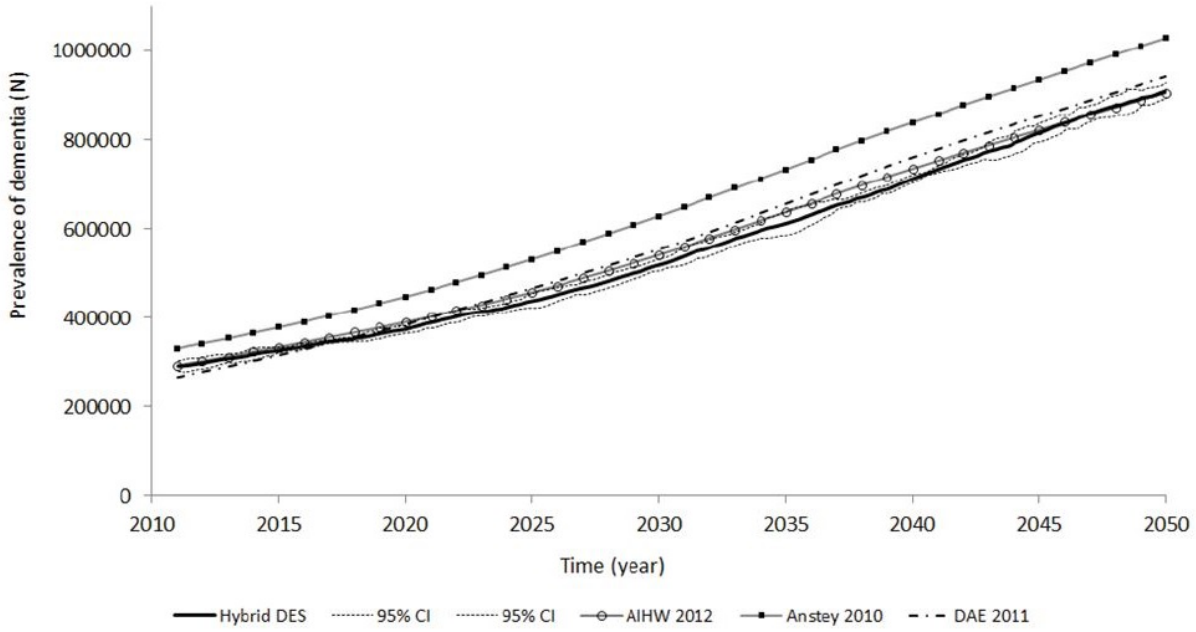

Figure 2: Comparison of dementia prevalence estimations in Australia: DES modelling by Standfield, *et al.* [7], AIHW[8] and Deloitte Access Economics[9]. From Standfield, *et al.* [7]

1.1.2 Health economics of Alzheimer's disease

Our ageing population and increasing prevalence of AD and other dementias worldwide will have a substantial impact on health care expenditure. The global costs of dementia are rising, from US $604 billion (2010) to US $818 billion (2015)[11]. In 2018, the total estimated worldwide cost of dementia was US $1 trillion and this will rise to US $2 trillion by 2030[11]. Although the prevalence of dementia is higher in lower middle-

income countries (Figure 1), approximately 90% of costs were incurred in high-income countries[11]. This unequal distribution of financial burden is even more pronounced when stratified according to membership to the G7 (funding 62% of global costs) or G20 (funding 92% of global costs)[11].

When compared with lower middle-income countries, high-income countries report higher per person costs of dementia, reflecting higher wages and lower proportion of care provided by informal unpaid carers. Wimo*, et al.* [11] divide the global estimates of dementia expenditure into three cost subcategories: direct medical costs, direct social care costs and costs of informal care. Since 2010, the distribution of costs between these subcategories has remained similar; however, as country income level increases there is an increasing relative cost contribution of direct social care and decreasing relative cost contribution of informal social care[11].

Alzheimer's Australia (AA) estimate the cost of dementia in Australia was AUD $14.25 billion in 2016, equating to on average AUD $35,550 per individual with dementia[12]. According to AA, this number will increase by 81% to AUD $25.8 billion in 2036 and AUD $36.8 billion by 2056[12]. This estimate from Brown*, et al.* [12] included costs borne from direct, indirect and intangible sources:

i) Direct costs: resources expended on an individual with dementia, for example aged care, hospital and specialist services or out-of-pocket costs borne by caregivers, family and friends in their social sphere
ii) Indirect costs: resources expended through a loss of productivity by the individual with dementia or their caregiver, for example the foregone earnings of an individual with younger onset dementia due to early retirement or those for a caregiver for loss of hours worked

iii) Intangible costs: non-material resources, for example the cost of pain, suffering, emotional toll, stress, exhaustion, isolation and fear that impact the burden of disease and contribute to the economic impact of the disease

Almost 85% of the costs attributable to dementia are related to family and social support, rather than medical care[13]. This burden can be seen to ripple throughout the family and social sphere, in turn having a negative health impact on the surrounding caregivers. Evidence indicates that people who care for a person with dementia are prone to mental disorders such as depression and anxiety[14]. The economic burden of AD is felt throughout society, therefore it has been classified by the World Health Organization (WHO) as a public health priority.

1.1.3 Impact on people, community

Living with AD perpetuates a substantial burden to the individual and their wider social sphere. Dementia has a profound impact on the individual as well as their relatives and other supporters, who have to respond to their increasing dependency. When compared with other chronic conditions, people with dementia require supervision and care from much earlier stages of disease progression. People with dementia often have comorbidities that intensify the level of care required. Alzheimer's Disease International (ADI) describe the impact of dementia as being visualized at three inter-related levels; the individual, who experiences ill health, disability, impaired quality of life and reduced life expectancy; the family/friends, who are the cornerstone of care and support and; the wider society, which either directly, or indirectly, incur the cost of health care and the loss of productivity[15]. To reduce the burden placed on the individual, the family/friends and wider society, action is required at all of the three inter-related levels. Current evidence indicates that manifestations of dementia are manageable, and while the underlying disease lacks a cure, it may be modifiable with evidence-based

management. The *2017 Lancet Commission on Dementia Prevention, Intervention, and Care* describes how effective dementia care, prevention and intervention could transform the future for wider society and greatly improve the lives of those in the sphere of dementia[13].

1.2 Clinical and neuropathological features of Alzheimer's disease

AD is a complex neurological disorder characterised by severe dendritic and synaptic loss, leading to memory impairment, language deterioration and visuospatial dysfunction. AD was initially documented in 1906 by Alois Alzheimer, who observed behavioural changes and cognitive decline in one of his patients, Auguste Deter[16]. Auguste's mental status deteriorated in her 50's, she became aggressive, delusional and was unable to remember recent events. Following the death of Auguste in 1906, Alzheimer performed an autopsy on Auguste's brain and discovered a diminished cerebral cortex, altered neurofibrils and neuritic plaques [17]. The discovery of this pathology is still relevant to the modern scientific community, whereby the distinctive pathological hallmarks are the deposition of extracellular deposits of the beta-amyloid (Aβ) protein, the abnormal intracellular phosphorylation of tau into neurofibrillary tangles (NFT), as well as cerebral atrophy and neuronal & synaptic loss. The behavioural symptoms of AD correlate with the accumulation of NFTs and neurodegeneration[18]. Currently, research suggests the progression of AD follows a temporal order of abnormal biomarker change, followed by the clinical manifestation of the disease[19].

The definition and diagnostic criterion of AD has undergone substantial change since the disease was initially documented via autopsy by Alzheimer. The AD field is fortunate to have three key neuropathological biomarkers that signal the progression of the disease: Aβ deposition, pathological tau tangles and neurodegeneration[20]. For this

reason, the National Institute on Aging-Alzheimer's Association (NIA-AA) Research Framework classifies AD biomarkers according to the AT(N) system, whereby A = amyloid, T = tau and N = neurodegeneration[20].

Although neuropathological features are hallmarks of disease progression, it is important to note that biomarker evidence of AD is not enough to diagnose. An individual may have extensive accumulation of Aβ and tauopathy yet display no cognitive deficits. Furthermore, an individual with neuropathological biomarker evidence of AD may not progress to MCI and dementia due to AD. To address this divergence between clinical symptomatology and pathophysiology, the NIA-AA convened to define the "preclinical" phase of AD[21]. The preclinical stage of AD precedes MCI and encompasses the spectrum of individuals who are clinically presymptomatic, yet display biomarker evidence for greater risk of disease. The NIA-AA developed the hypothetical model of the pathological-clinical continuum of AD (Figure 3).

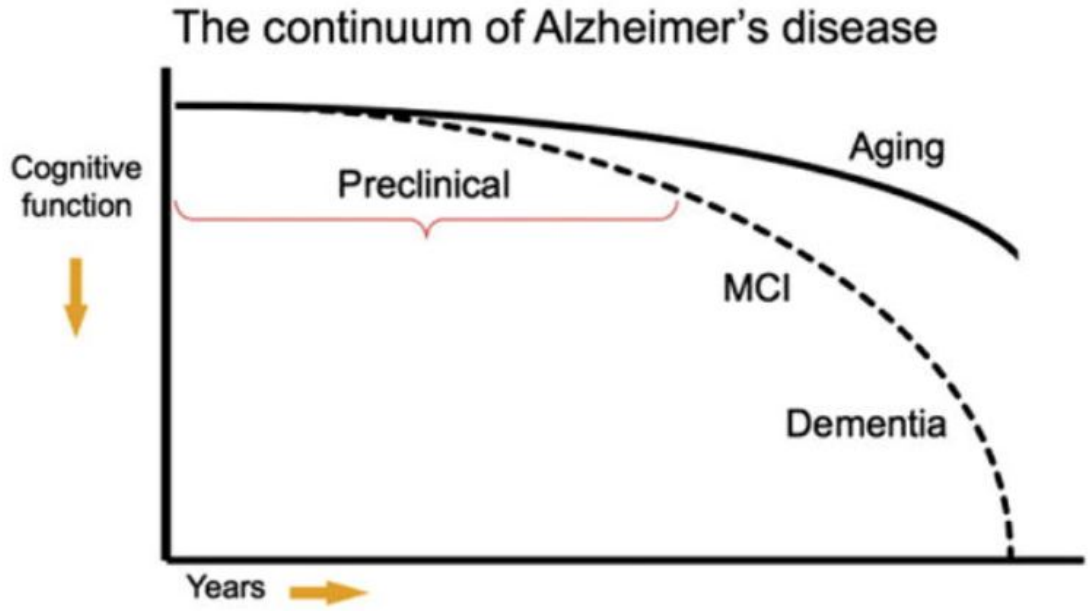

Figure 3: Modelling the cognitive trajectory of Alzheimer's disease. From Sperling, *et al.* [21]

Along the continuum of AD progression, the preclinical phase represents an ideal window for intervention. Evidence from clinical trials, basic science and epidemiological research indicates that therapeutic interventions applied earlier in AD progression would be more likely to achieve disease modification. A hypothetical intervention that delayed AD onset by only five years could reduce the number of patients with AD dementia by 57%[21]. Thus the utility of biomarkers is not only in their ability to predict disease progression, but their potential to discover disease modifying therapies in the preclinical phase of the AD continuum.

1.2.1 Beta-amyloid plaques

Extracellular accumulation of Aβ is the first pathological hallmark of AD. The pathological aggregation of Aβ plaques is implicated in the synaptic and dendritic degradation that leads to cognitive decline in AD. The pathway of Aβ is predominantly extracellular, occurring outside the neuronal membrane. Aβ, or beta-amyloid, refers to an array of hydrophobic peptides of 39-43 amino acids, predominantly $A\beta_{40}$ and $A\beta_{42}$ that are derived from the much larger amyloid precursor protein (APP) in a proteolysis reaction involving two membrane-bound enzyme complexes, β- and γ-secretase[22]. β-secretase cleaves the N terminus of the Aβ domain of APP, generating secreted APP-β as well as a membrane bound fragment (C99) that contains the entire Aβ domain. γ-secretase then further cleaves this fragment, generating Aβ peptides of varying lengths. These peptides aggregate into self-assembled macrostructures with characteristic supe-secondary reverse turn structures (Figure 4). Protofibrils formed by aggregated Aβ disrupt normal tissue architecture and damage the surrounding neurons and go on to form fibrils that deposit in specific regions of the brain[23]. The star shaped masses of Aβ fibrils form the plaques that are characteristic of AD. Given their integral role in the Aβ aggregation pathway, β- and γ-secretases have become potential targets for drugs to reduce Aβ production and increase Aβ clearance. Pharmacological treatments, including

monoclonal antibodies, are designed to act upon every step of the pathway; from early monomer aggregation to eventual plaque formation.

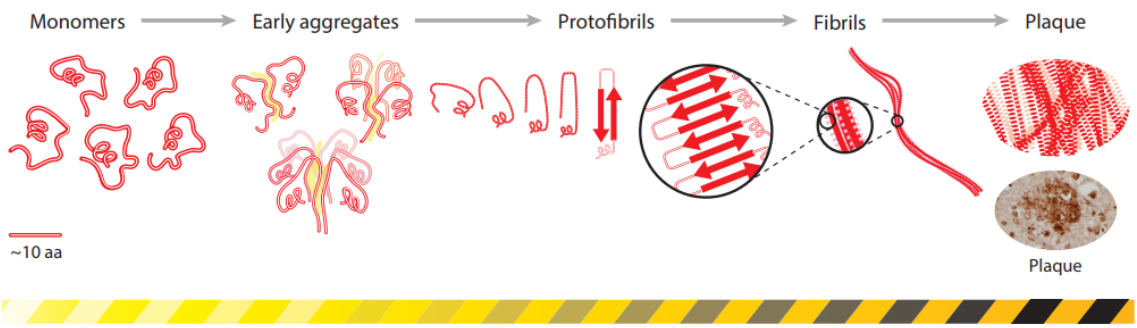

Figure 4: Pathway of monomer-plaque formation of beta-amyloid protein in Alzheimer's disease. Modified from Graham, *et al.* [23]

Due to discoveries involving APP mutations and presenilin, the amyloid cascade hypothesis suggested that the pathway of Aβ aggregation pictured in Figure 4 was the primary cause of AD. Although the failures of pharmacological interventions suggest the Aβ pathway is not fully understood, the prevailing theory of amyloid aggregation places Aβ upstream of all other AD neuropathological change. The amyloid cascade hypothesis[24,25] posited that Aβ deposition and aggregation was the causative agent of AD neuropathology and that NFTs, neuronal loss, vascular damage and clinical symptomatology were a direct result of this deposition. The theory provided by Hardy, *et al.* [24] proposed the overproduction, or clearance reduction, of Aβ was the underlying cause of cognitive decline in AD. Through a series of downstream events (Figure 5), Aβ accumulation followed by oligomerisation leads to altered microglial and astrocytic activation[23]. Alterations in neuronal ionic homeostasis are understood to be the root of oxidative injury, leading to the altered kinase/phosphatase activities that form NFTs[23]. Dendritic and synaptic dysfunction cause the neuronal loss that is believed to be the root of clinical AD.

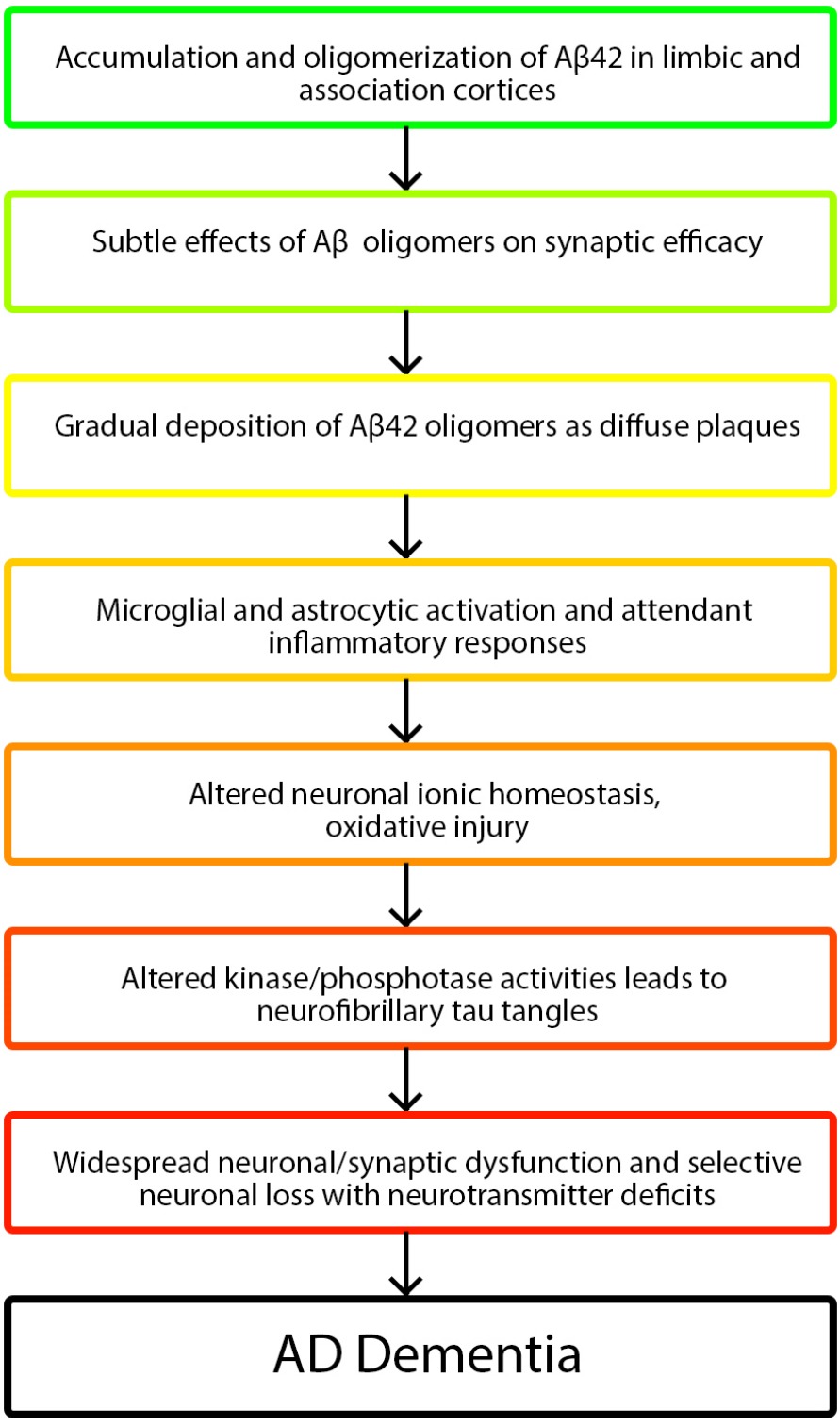

Figure 5: The Amyloid Cascade Hypothesis[24]: Pathway of major pathological events leading to AD dementia. Created by E.Hill, modified from Selkoe, *et al.* [26]

1.2.2 Neurofibrillary tau tangles

Intracellular NFTs of tau are the second hallmark biomarker of AD. NFTs begin with monomer isoforms of the microtubule association protein (MAP) tau. In normal neurons, the phosphoprotein tau enhances the polymerisation of tubulin to microtubules. In an altered state, tau is excessively phosphorylated due to enhanced activity of kinases and decreased activity of phosphatases. The hyperphosphorylation of tau, whether caused by mutation, proteolysis, polyamines or a combination of factors, causes assembly of soluble tau aggregates (Figure 6). These aggregates form paired helical filaments that go on to form NFTs, leading to cytoskeletal disruption along the axon, eventually causing cell death.

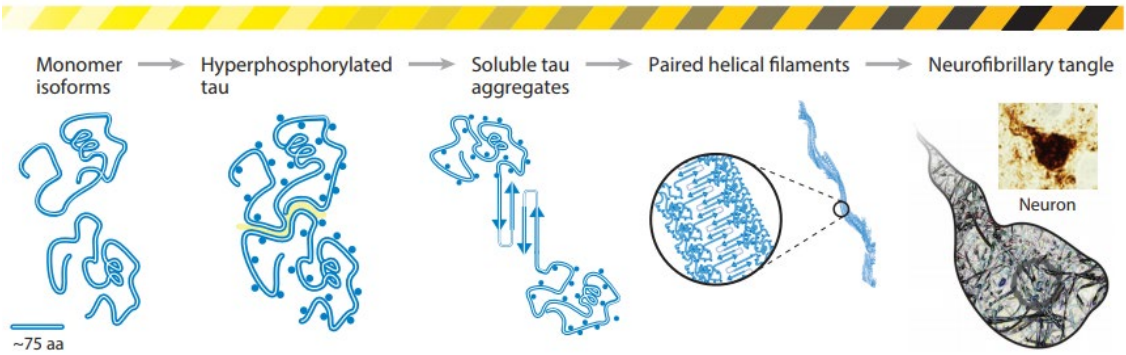

Figure 6: Pathway of monomer-plaque formation of tau neurofibrillary tangles in Alzheimer's disease. Modified from Graham, *et al.* [23]

1.2.3 Neurodegeneration

After Aβ and NFTs, neurodegeneration is the third neuropathological correlate of AD. Neurodegeneration, or neuronal injury, refers to the progressive loss of structure and function in neurons, including eventual cell death. According to the prevailing amyloid cascade hypothesis, neurodegeneration occurs downstream of the two other neuropathological features of AD: Aβ accumulation and NFTS of the MAP tau. The process of neurodegeneration in AD is initially characterised by synaptic damage leading to neuronal loss and has more recently been suggested to be affected by altered

neurogenesis in the hippocampus[27]. Neurodegeneration in AD, particularly synaptic loss, has correlated strongly with cognitive symptoms[28]. The loss of synapses, axonal neuropathology and altered neurogenesis are likely key neuropathological features that lead to AD dementia, representing a multi-pronged attack on the brain (Figure 7)[27].

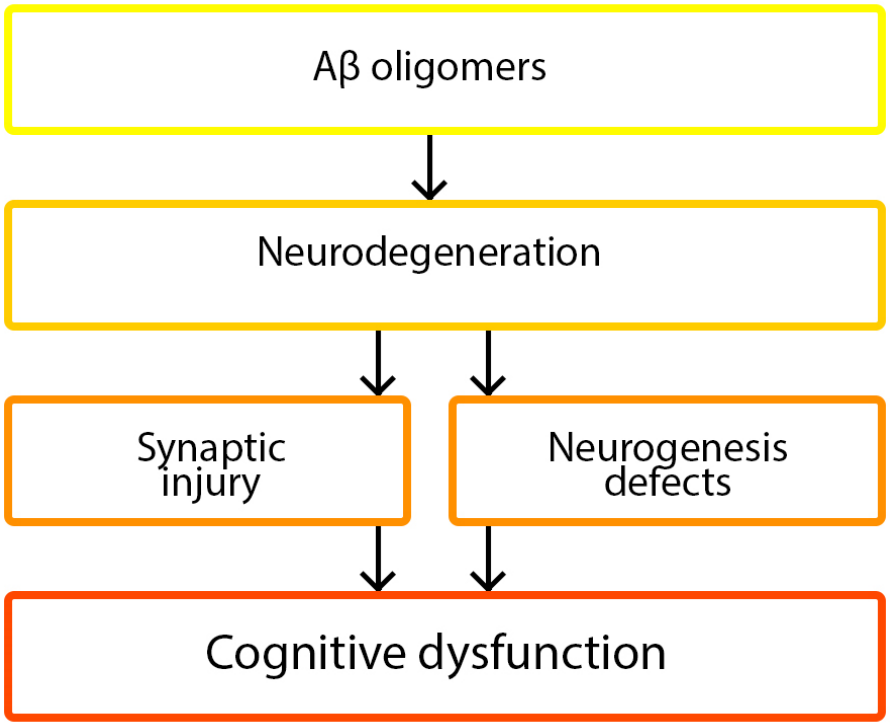

Figure 7: Mechanisms of neurodegeneration in AD. Created by E.Hill, modified from Crews, *et al.* [27]

Disturbed synaptic integrity and neurogenesis display strong correlates to the cognitive impairment seen in AD[27]; yet the prevailing dogma in the field suggests the direct abnormal accumulation of Aβ oligomers located in the nerve terminals precedes synaptic damage that ultimately leads to neurodegeneration. Subsequently, the focus on AD pharmacological therapies in recent years has been to improve memory by reducing Aβ or tau deposition. Although current pharmacological therapeutics lack efficacy to

reduce disease progression, several mechanisms target reducing Aβ accumulation to improve cognitive function[29]:

iv) Anti-aggregation molecules that block fibrils and oligomers

v) APP regulators that block both the β- secretase and γ-secretase

vi) APP regulation through lipid and/or cholesterol modulation

vii) Down-regulation of APP through small interfering ribonucleic acids (siRNA)

viii) Increasing Aβ clearance with antibodies, APOE, lysosomal/proteasomal pathways

1.2.4 Clinical symptomatology

Together with a neuropathological profile of Aβ, NFTs and neurodegeneration, AD manifests in clinical symptomatology, known as mild cognitive impairment (MCI) or dementia. Clinical symptoms of AD are believed to be preceded by up to decades of neuropathological change and the subject-specific time lag between biomarker evidence of AD and the emergence of cognitive impairment is likely mediated by brain resiliency or cognitive reserve[30]. Based on plethora research findings into AD biomarker correlates, the hypothetical model of AD progression developed by Jack*, et al.* [19] places clinical symptomatology, or cognitive impairment, as the final stage of disease progression (Figure 8).

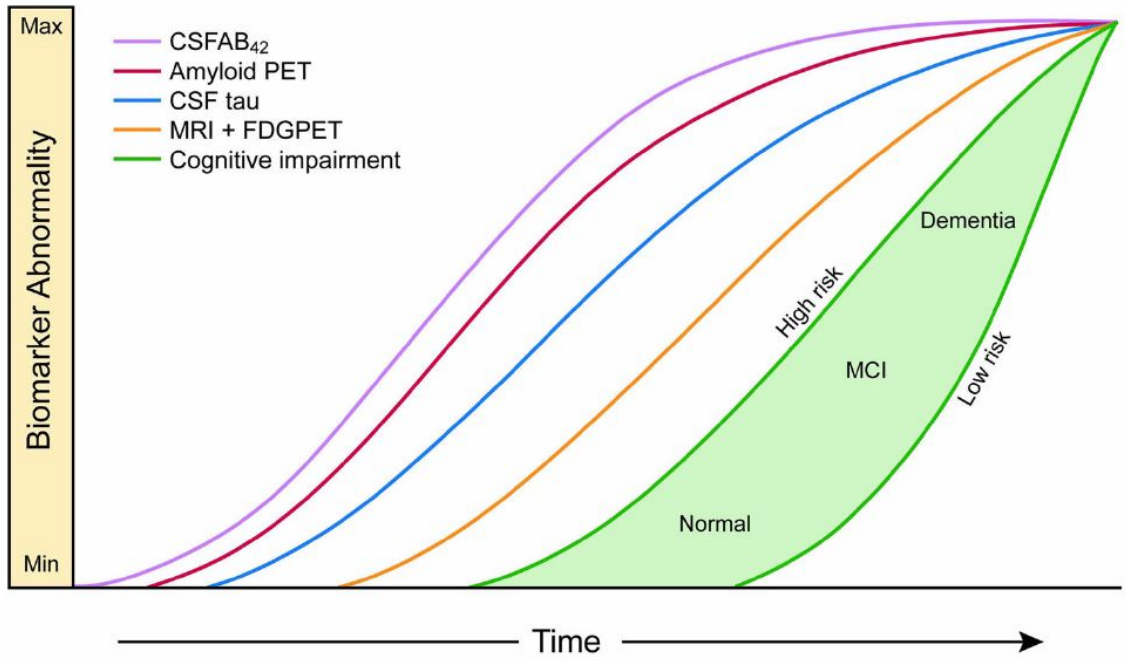

Figure 8: Updated hypothetical model of dynamic biomarkers in the AD neuropathological cascade. From Jack, *et al.* [19]

In 2011, the NIA-AA recognised clinical AD progression was along a continuum rather than three distinct clinically significant categories[31]. This notion has been formalised and updated in the 2018 NIA-AA research framework to conceptualise an individual's progression of clinical symptoms from cognitively unimpaired to dementia. From cognitively unimpaired (CU) to dementia, an individual's cognitive performance, activities of daily living and nonbehavioural symptoms are placed along a cognitive continuum, then classified as either: CU, MCI or dementia (Table 1).

Category	Syndromes along cognitive continuum
Cognitively Unimpaired (CU)	- Cognitive performance within age-adjusted and sex-adjusted expected range (based upon all available normative data, clinical judgment and demographic information)
	- Cognitive performance within impaired/abnormal range, yet performance is within the range expected for that individual (based upon all available normative data, clinical judgment and demographic information)
	- A subset of CU individuals may report subjective cognitive decline or demonstrate subtle decline in cognitive testing
Mild Cognitive Impairment (MCI)	- Cognitive performance below age-adjusted expected range (based upon all available normative data, clinical judgment and demographic information)
	- Cognitive performance is usually within impaired/abnormal range, but this is not required given they are below the range that is expected of that individual
	- In addition to evidence of cognitive impairment, evidence of decline from baseline must be present (either from researcher, clinician, study partner or individual)
	- Although cognitive impairment/decline is core criteria, neurobehavioral disturbance may be a prominent feature
	- Activities of daily life performed independently, but cognitive difficulty may result in more complex activities
Dementia	- Substantial progressive cognitive impairment that affects several domains and/or neurobehavioral symptoms (reported either from researcher, clinician, study partner or individual)
	- Cognitive impairment and/or neurobehavioural symptoms result in clear functional impact on activities of daily life. No longer independent. May be subdivided: mild, moderate, severe

Table 1: Along the cognitive continuum of clinical categories. Modified from Jack, *et al.* [20]

1.2.5 Diagnosing Alzheimer's disease

Autopsy is required to definitively diagnose AD; however, a research framework exists to aid in understanding disease progression *in vivo*. Given 10% to 30% of individuals clinically diagnosed as AD dementia lack an AD neuropathology at autopsy[32], the NIA-AA recommends understanding AD within a research framework, as opposed to a

definitive diagnosis. Clinical features, as well as neuropathological change (AT[N]) provide the foundation for an AD, or probable AD, diagnosis (Figure 9).

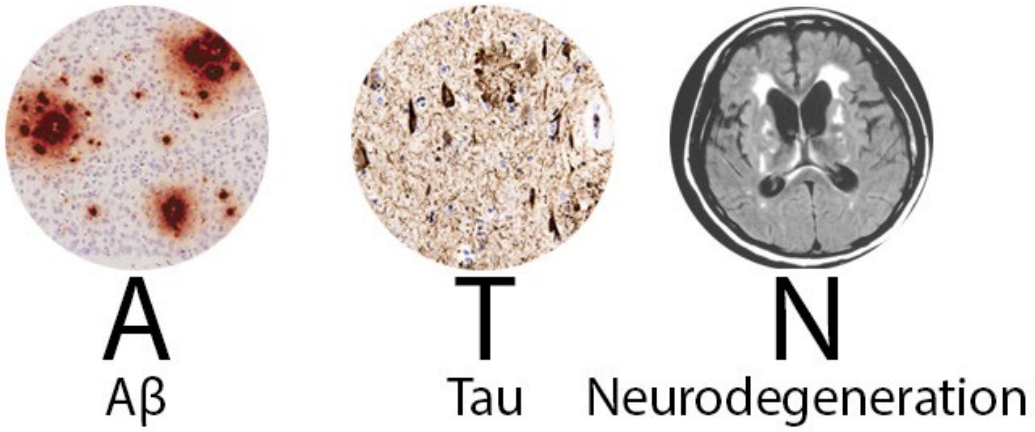

Figure 9: NIA-AA AT(N) categories of AD diagnosis[20]. A (Amyloid-Beta), T(tau) and N (Neurodegeneration). Created by E. Hill

AD is often diagnosed clinically using criterion within the NIA-AA[31], the International Classification of Disease (ICD-10[33]), Diagnostic and Statistical Manual of Mental Disorders V Edition (DSM-5[34]), the International Working Group (IWG[35]) and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA[36]). Although they display a high degree of diagnostic agreement[37], clinical diagnoses also require exclusion of co-occurring medical conditions that differentiate between these criteria. Currently both the NIA-AA[20] and IWG[35] have incorporated neuropathological biomarkers into their diagnostic guidelines for AD. This represents a shift in focus of AD diagnosis, from a clinical syndrome to a biological construct.

According to the recently updated NIA-AA research framework, an AD neuropathological diagnosis on the AD continuum requires placing an individual into the AT(N) categories (Section 1.2). Binarizing the three AT(N) biomarker types leads to eight different AD biomarker profiles (Figure 10). An individual must be amyloid

positive (A+) to fall along the Alzheimer's continuum. However, an individual may be tau positive (T+) and display neurodegeneration (N+), yet not be classified along the Alzheimer's continuum and instead be classified as displaying non-AD pathological change.

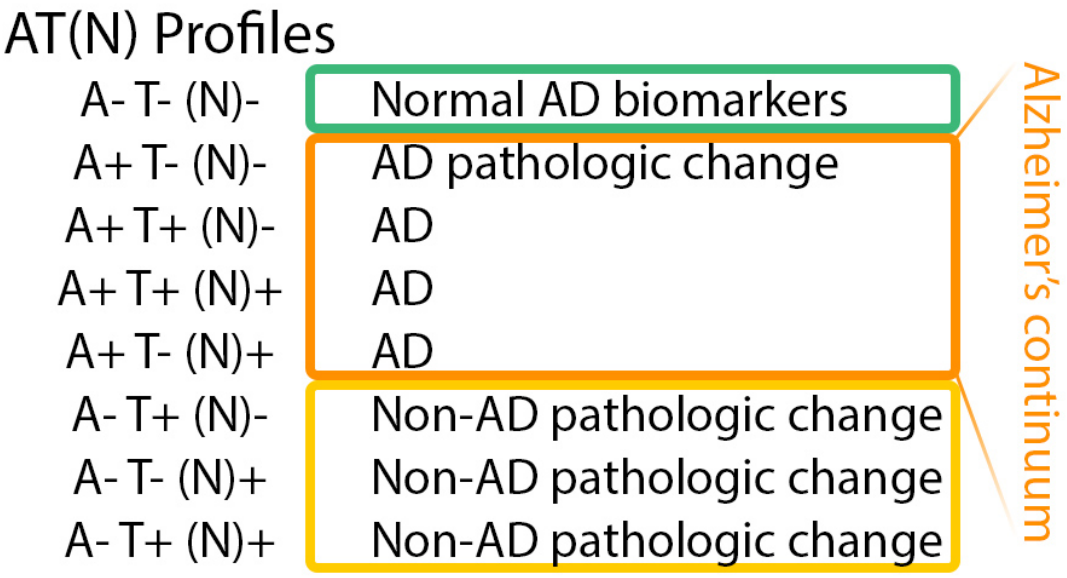

Figure 10: Eight biomarker profiles from binarization of NIA-AA AT(N) biological criteria for the diagnosis of Alzheimer's Disease. Created by E.Hill, modified from Jack, *et al.* [20]

The measurement of neuropathological change according to the NIA-AA AT(N) categories is undertaken through several different methods. To date, the most widely utilised and validated *in vivo* biomarkers of amyloid Aβ and tau are positron emission tomography (PET) imaging, cerebrospinal fluid (CSF) and plasma assays. Structural magnetic resonance imaging (MRI) is also utilised to visualise neurodegeneration in the form of brain atrophy in areas such as the medial temporal lobes, paralimbic, temporal and parietal cortex.

Recent advances in amyloid imaging have allowed *in vivo* quantification of Aβ aggregation to aid in diagnoses. PET has been increasingly utilised to capture images of cerebral Aβ deposition and the validity of Aβ PET radiotracers for the diagnosis of AD

has been supported by systematic review[38]. Results from Aβ PET studies have been critical to the discovery that deposition of Aβ in the brain commences 15-20 years prior to clinical diagnosis[39]. Cerebral glucose metabolism, a proxy for neuronal activity, has also been measured by PET using the ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) radiotracer[40] and a similar method is utilised for tau PET with the AV1451 radioligand[41]. To detect cerebral Aβ burden, a radiotracer or radioligand must be a small molecule capable of crossing the blood-brain barrier (BBB) and adequately binding to the appropriate Aβ constituent, for example ^{18}F florbetaben binds specifically to Aβ plaques (Figure 11). Recently, ^{11}C and ^{18}F labelled radiotracers were designed to display high *in vivo* binding to Aβ: N-methyl-[11C]2-(4=-methylaminophenyl)-6-hydroxybenzothiazole (Pittsburgh Compound B, or [^{11}C]PIB), flobetapir ([^{18}F]AV-45), flutemetamol ([^{18}F]GE067) and florbetaben ([^{18}F] BAY94–9172)[42] (Figure 12). Aβ PET imaging begins with the injection of the radioactive tracer into the patient. After a short time, depending on the specific radioactive compound's binding affinity, specificity and half-life ([^{18}F] approximately 110 minutes; [^{11}C] approximately 20 minutes[43]), the distribution of cerebral deposition is quantified using PET scanning. PET detects subatomic particles, positrons, emitted by the ^{18}F/ ^{11}C isotope when they collide with an electron and produce gamma radiation through an annihilation reaction[44]. Three dimensional images of the radiotracer are then reconstructed utilising iterative computer software techniques, motion-corrected and smoothed to a three dimensional plane. Regional and whole cerebellar standard uptake value ratios (SUVR) are calculated by normalising regional uptake, correcting for atrophy evident in structural MRI. Neocortical SUVR, a global measure of Aβ aggregation, is calculated by averaging regional SUVRs by their area-weighted means. Various methods have been utilised for defining the neocortical global SUVR cut-off point for a neuropathological diagnosis of AD in living persons[45]. After examining five methods for determining cut points in Aβ

PET, tau PET, FDG PET and MRI cortical thickness, Jack Jr, *et al.* [45] defined a ^{18}F PET SUVR of 1.42 [centiloid 19] to binarize an A-/A+ neuropathology.

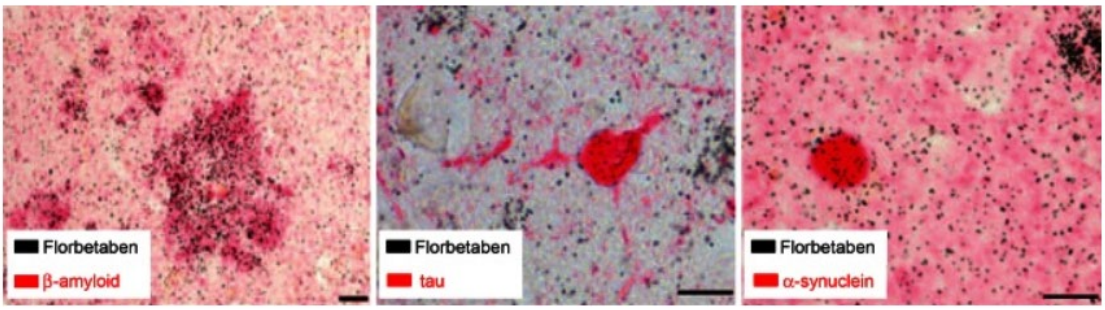

Figure 11: Specific binding of ^{18}F Florbetaben to Aβ plaques. This image was created using autoradiography using immunohistochemical staining for Aβ (left), tau (middle) and α -synuclein (right) in post-mortem human brain slices. ^{18}F Florbetaben correlates with the Aβ only. From Sabri, *et al.* [46]

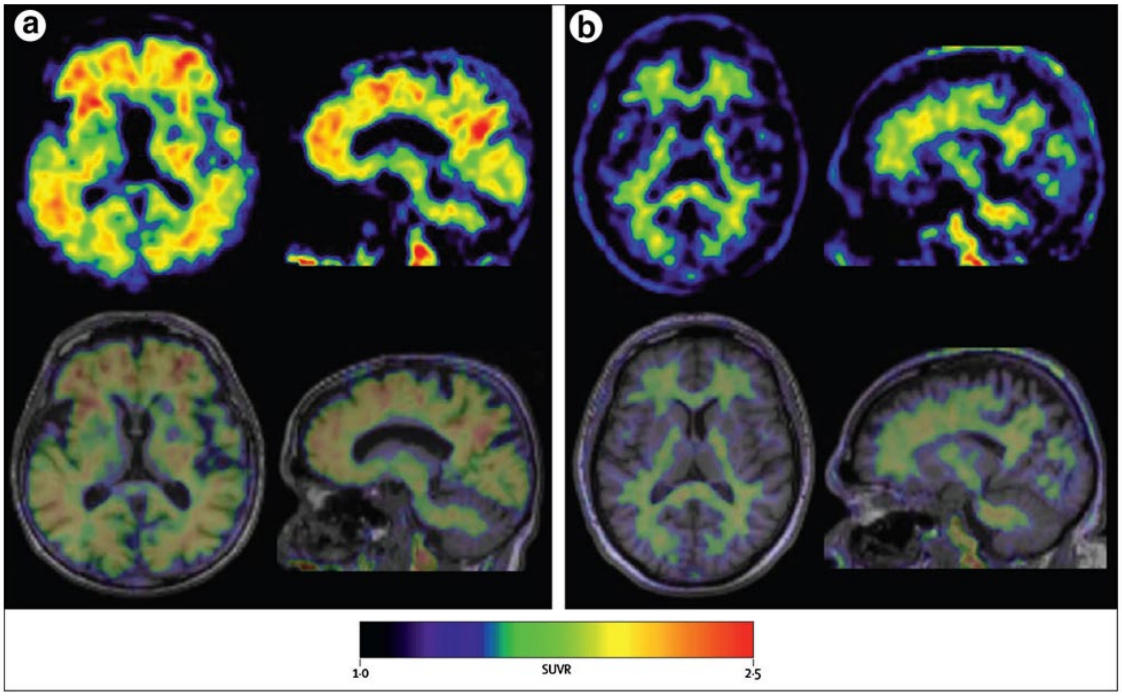

Figure 12: ^{18}F Florbetaben to predict cerebral Aβ burden. PET images (upper) and PET/MRI (lower) show a) AD dementia patient and b) Healthy control. From Sabri, *et al.* [46]

CSF assays have also been utilised in the identification of AD, showing a similar accuracy in diagnosing early-stage AD to amyloid PET[47]. CSF is usually collected via invasive lumbar puncture between vertebrae in the lower spine and analysed using enzyme-linked immunosorbent assay (ELISA). ELISA batch analysis can be utilised to measure plethora of biomarkers in AD, such as total tau (t-tau), phosphorylated tau (p-tau), Aβ40, and Aβ42. CSF Aβ42 inversely correlates with cerebral Aβ load and CSF t-tau correlates with neocortical NFTs[48,49]. Research suggests that cerebral and peripheral pools of Aβ are in equilibrium and that changes in CSF levels of AD biomarkers may reflect an altered neuropathology. CSF collection and ELISA analysis are also substantially cheaper options for diagnosing AD pathology than PET scans[50].

Recently, the utility of plasma as a minimally invasive, cost-effective blood-based biomarker for AD has been described. With independent datasets in Australia and Japan, Nakamura*, et al.* [51] showed plasma Aβ could predict cerebral Aβ burden with high accuracy. Plasma levels of circulating Aβ were also significantly correlated with CSF Aβ[51]. Given the diagnostic accuracy and cost-benefits of plasma AD assays, further validation studies and standardised operating procedures are required before this method overtakes PET scans as the most common method of diagnosis.

1.3 Risk factors for Alzheimer's disease

Currently AD lacks a disease-modifying treatment and research has shifted focus toward investigating delaying or preventing the onset of the clinical (dementia) and neuropathological (AT[N]) features of the disease. Research from international epidemiological studies suggests a reduction in dementia risk due to an individual's lifetime exposure to health and lifestyle factors. Advances in biomarker measurement have permitted epidemiological research to elucidate the potential brain mechanisms that impact therapeutic efficacy in modifiable lifestyle risk factors for AD. In order to minimise the incidence, prevalence, economic and social burden of AD, decreasing an individual's exposure to risk factors, whilst increasing their exposure to protective factors is a global priority.

In 2017, *Lancet Commission on Dementia Prevention, Intervention and Care* met to discuss emerging knowledge and to consolidate the evidence regarding dementia prevention[13]. Neuropathological findings suggest multiple mechanisms of action that display therapeutic potential (Figure 13), whether individually or synergistically.

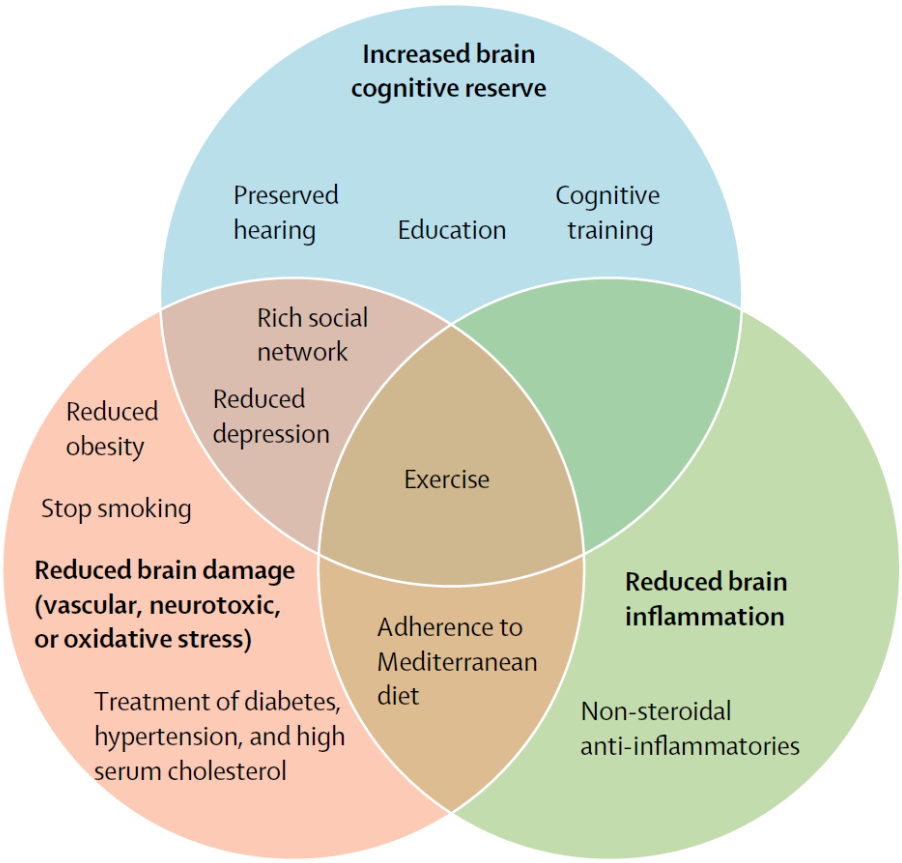

Figure 13: Potential brain mechanisms for modifiable lifestyle risk factors. From Livingston, *et al.* [13]

With an abundance of research for modifiable lifestyle factors, such as smoking, exercise and diet, being related to dementia prevention, research is investigating the preventative potential of individual or multi-modal interventions. In 2014, Norton*, et al.* [52] posited approximately one third of AD cases could be attributed to potentially modifiable risk factors. The *2017 Lancet Commission on Dementia Prevention, Intervention and Care* utilised international epidemiological findings to construct a life-course model of the contribution of modifiable risk factors to dementia risk (Figure 14).

The *Lancet* model estimated 35% of dementia risk could be attributed to modifiable lifestyle risk factors throughout the life-course, such as education in early life, hearing loss in mid-life and smoking in later life[13]. Although the majority of dementia cases are attributed to non-modifiable risk factors, such as the greatest risk factor; age, there is a

substantial proportion of disease burden that is potentially modifiable. Assuming the true proportion of Alzheimer's disease cases attributable to modifiable lifestyle risk factors lies between 28.2%[52] and 35.0%[13], there is a clear incentive for investigating the potential of these risk factors to reduce the global incidence of AD.

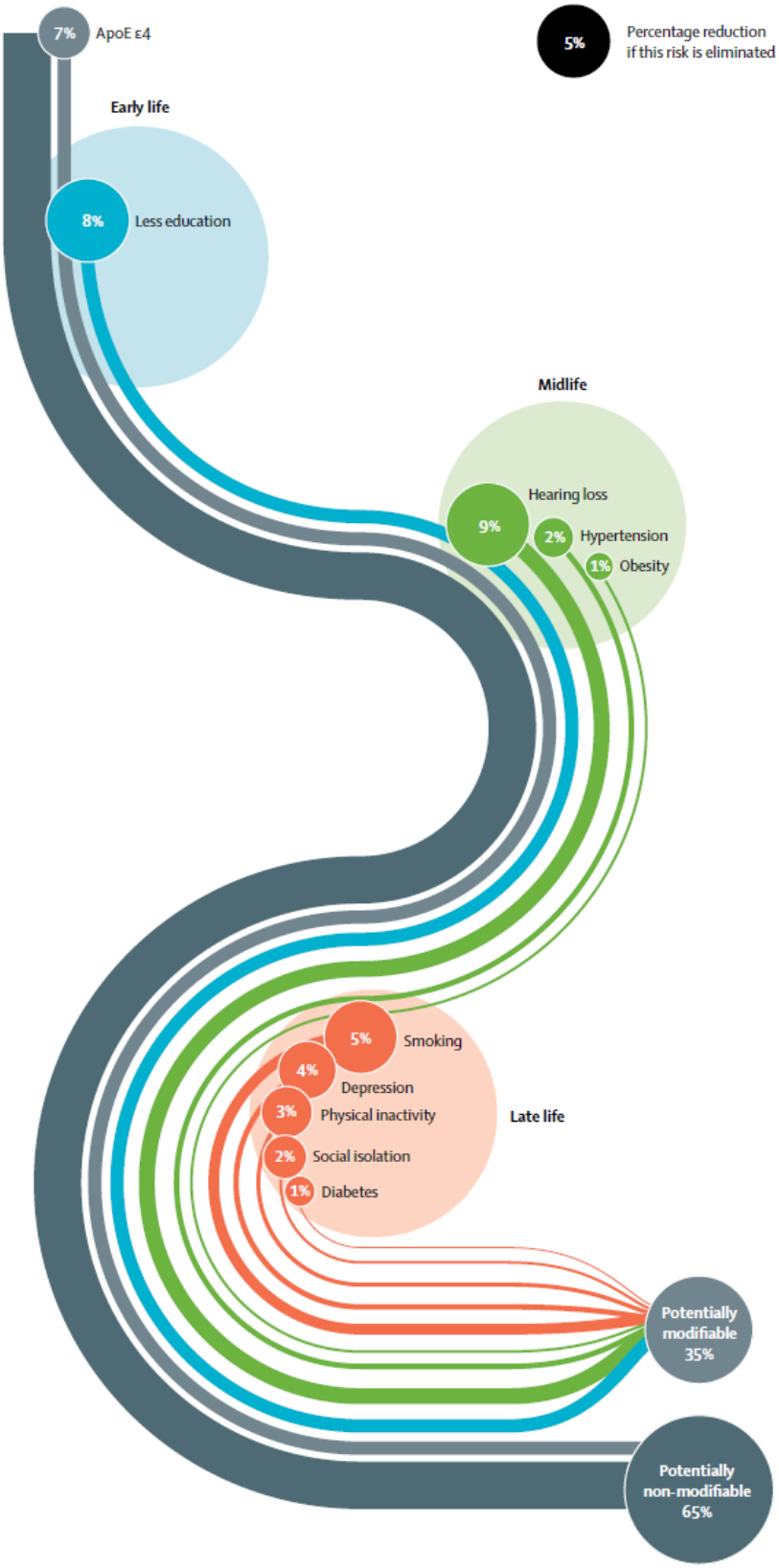

Figure 14: Life-course model of potentially modifiable and non-modifiable risk factors for dementia. From Livingston, *et al.* [13]

1.3.1 Non-modifiable

1.3.1.1 Age

Ageing is the biggest risk factor for dementia; however, dementia is not an inevitable consequence of ageing[13]. Usually dementia occurs in individuals aged over 65 years, with approximately 80% of dementias in individuals aged 75 years or older[53]. Dementia prevalence increases by a factor of two with every 5 years of ageing, equating to approximately 1% incidence at 60 years and 30% incidence at 85 years of age[54,55].

1.3.1.2 Genotype

Many individuals have a genetic predisposition for AD, with several genetic profiles identified as inferring a greater risk for the disease. The major genetic risk factor, apolipoprotein E epsilon 4 (APOE-ε4), has been calculated to contribute 7% of AD incidence[13]. Peripheral ApoE is mainly synthesised in the liver and is involved in lipid/cholesterol transport[56]. Within the central nervous system (CNS), ApoE is a major lipoprotein synthesised and secreted from astrocytes and microglia[57].

ApoE appears to be necessary for Aβ deposition and clearance in the brain[58] and is associated with two single-nucleotide polymorphisms that mark three alleles (ε2, ε3, ε4). The three APOE isoforms, denoted as APOE-ε2, APOE-ε3 and APOE-ε4, have varying distributions in the Caucasian population at 8%, 78% and 14% respectively[59]. Research suggests the ε2 isoform may offer a level of protection against AD and conversely carrying one or two of the ε4 alleles incrementally increases an individual's risk of disease development[60]. People who are homozygous for the APOE-ε4 allele (ε4/ ε4) are at a much higher risk of developing AD than those homozygous for APOE-ε2 allele (ε2/ ε2), implying the magnitude of risk is in the order ε4/ ε4 > ε4/ ε3 > ε4/ ε2 or ε3/ ε3 > ε3/ ε2 > ε2/ ε2. Heterozygous individuals on the ε4 allele (ε4/ ε3 or ε4/ ε2) have three times the risk of developing sporadic AD, whilst this increases to fourteen times the risk for homozygous carriers (ε4/ ε4) [61,62].

Epidemiological and basic research supports the impact of APOE on the clinical and neuropathological features of AD. Meta-analysis has revealed a significant effect of APOE on cognition and the strength of this association was more pronounced in older age groups[63]. In mice models, APOE knockouts crossed with a mice model for AD display considerably less Aβ deposition[64,65].

Several other genetic markers have been related to AD. Over 80% of individuals with early-onset AD (>60 years of age) carry autosomal mutations in one of three genes: APP on chromosome 21, Presenilin-1 (PSEN-1) on chromosome 14 or Presenilin-2 (PSEN-1) on chromosome 1[66]. APP encodes the Aβ precursor protein that is processed by β- and γ-secretases leading to the production of Aβ[67]. PSEN-1 and PSEN2 encode the presenilins, constituting the catalytic subunit of the γ-secretase complex[68,69].

1.3.1.3 Sex

Women are disproportionally affected by Alzheimer's disease than their male counterparts[70]; however, the underlying pathological processes are yet to be determined. Two-thirds of the people with dementia due to AD are women, and women account for two-thirds of the unpaid caregivers of individuals with AD[71]. Globally, women with dementia outnumber men by a ratio of 2:1[72], and research indicates this discrepancy is due to several factors. Age is the biggest risk factor for dementia and women have longer life expectancies worldwide than men[72]. With greater longevity and prolonged exposure to various other risk factors, women have a higher prevalence for dementia across the globe regardless of income levels[73].

Depression, diabetes and obesity are all factors that differ by sex throughout life and are associated with an increased risk of AD[74]. Females that experience cumulative symptoms of depression have been suggested to have an increased risk for MCI and dementia in later life [75,76]. Sex differences in sleep disorders[77] may account for some of

the discrepancy in AD impact, as studies have found associations between sleep patterns and Aβ deposition in humans[78] and mice[79].

Males and females display neuroanatomical differences; the male brain is approximately 10% larger[80], the proportion of white/grey matter differs[81] and brain volume tends to decline faster in males than females[82]. Longitudinal studies of cognitive ageing demonstrate faster decline in males than females[83]; however, this is reversed in MCI/AD populations[84]. Studies have reported that female APOE-ε4 heterozygotes have greater risk for AD than male ε4 heterozygotes[85,86]. Some studies suggest that females with at least one APOE-ε4 allele are also more likely than males to convert from MCI to AD[87].

Post-menopausal women also have a higher risk for developing AD[88]; however, there is ongoing debate over the age-dependent stage of menopause[89]. Oestrogen is important for neuronal maintenance and brain function in the nucleus basalis of Meynert and other cerebral regions typically affected by AD[88]. Oestrogen is suggested to exert a neuroprotective effect and has been shown to enhance the growth and survival of cholinergic neurons[90,91], increase cholinergic activity[92] and promote the nonamyloidogenic metabolism of APP[93]. During and after the menopausal period, oestrogen displays a dramatic decrease in peripheral blood therefore post-menopausal status is often considered an additional risk factor for AD[94]. Furthermore, the decrease in oestrogen has also been associated with an increase in Aβ[95].

1.3.2 Modifiable

1.3.2.1 Education

Projection models indicate that primary prevention, aimed at reducing the incidence of AD, is likely to delay disease onset and reduce the global prevalence[96]. Brookmeyer*, et al.* [97] estimated that delaying AD by only one year could reduce worldwide incidence in

people over 60 years old by 11%. In an analysis of meta-analytic reviews, Barnes*, et al.* [98] identified seven potentially modifiable risk factors (Figure 15) for AD, noting a 10-25% reduction in all seven risk factors could potentially prevent 3 million cases of AD worldwide. Approximately one in five cases of AD worldwide was estimated to be attributable to low educational achievement[98]. According to the cognitive reserve model, higher levels of education compensate for the neurodegeneration seen in AD, delaying clinical symptoms. Systematic review has noted an increase in education delays the onset of dementia[99] and that lower education was associated with a greater risk of dementia[100].

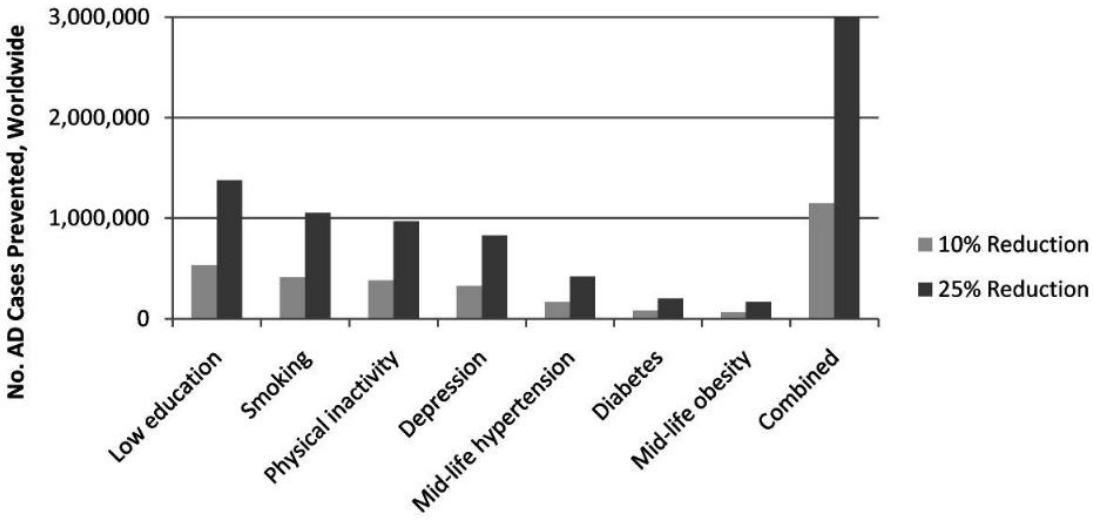

Figure 15: Potentially preventable AD cases through reductions in risk factors of 10% or 25% worldwide. From Barnes & Yaffe[98]

More recently, the *2017 Lancet Commission on Dementia Prevention, Intervention and Care* model estimated that 8% of dementia risk can be reduced if childhood education targets are met[13]. In later life, cognitive resilience is likely to be enhanced by laying foundational brain reserve earlier in life through education and intellectual stimulation[101,102]. A reduced risk for AD in highly educated individuals has been

proposed to reflect an increased cognitive reserve that heightens the brain's resilience to disease pathology and can therefore delay the clinical expression of the disease[30]. Cognitive reserve is posited to be associated with the cerebral anatomical substrate or cognitive adaptability[103,104]. An individual with higher reservoirs of cognitive resilience would therefore have a delayed risk for developing dementia. Results of two large scale studies[105,106] conducted in the United States (Framingham Heart Study and Health and Retirement Study) displayed an association between an increase in education and a decrease in the age-specific prevalence of dementia, despite an increase in the incidence with age.

With regards to the neuropathological features of AD that precede clinical manifestations, higher educational attainment has been associated with lower cerebral Aβ accumulation[107]. Education has also been suggested to enact a neuroprotective effect on AD neuropathology (total brain volume) in the longitudinal study: Alzheimer's Disease Neuroimaging Initiative (ADNI)[108]. Research has displayed that early intellectual enrichment may be associated with protection and compensation for later life Aβ accumulation[109].

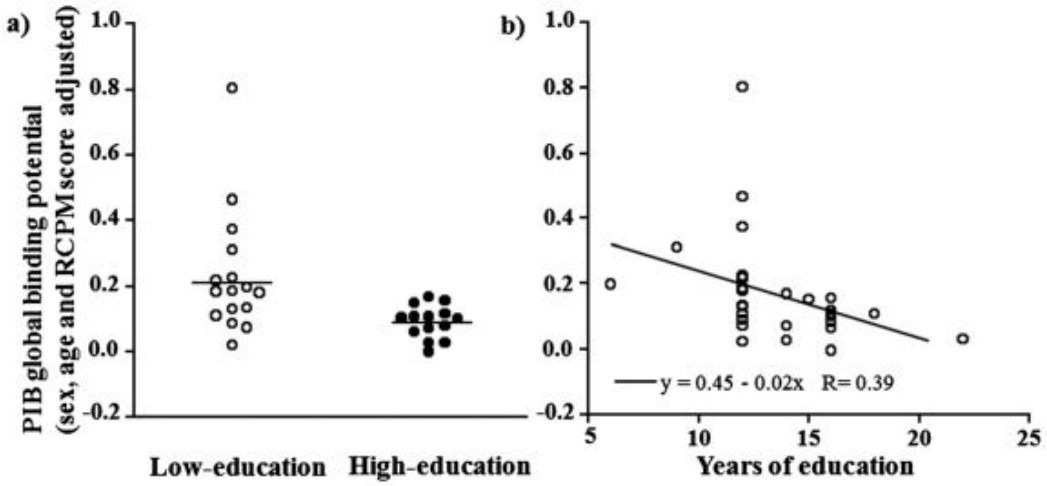

Figure 16: a) Scatter plot of global cortical mean Pittsburgh Compound B-Binding P potential (PIB-BP) of participants in the low/high education groups; b) Scatter plot of PIB-BP and duration of education (in years). Adjusted for age, sex and Raven's Colored Progressive Matrices (RCPM) score. From Yasuno*, et al.* [107]

1.3.2.2 Exercise

Engaging in exercise is helpful to reduce AD risk through a variety of mechanisms, including cardiovascular health, cerebrovascular health, diabetes and obesity. Epidemiological evidence suggests a strong association between increased levels of physical activity and protection against cognitive impairment in later life[110,111]. Although there has been systematic[112] and Cochrane reviews[113], the evidence from randomised control trials (RCT) regarding exercise interventions to improve cognitive outcomes is extremely variable. This may be due to the time-window of heightened physical activity, a physical activity intensity threshold[114] or a dose-response association between high-intensity exercise and cognition[115]. In women who reported physical activity at 30 years, 50 years and late life had a reduced risk of developing cognitive impairment in later life compared with women who reported physical inactivity[116]. This

effect was heightened in women who engaged in higher physical activity in their earlier years.

Exercise is believed to exert a neuroprotective effect on the underlying AD pathology, whether through reducing vascular risk, reducing cortisol or promoting the release of brain-derived neurotrophic factor (BDNF)[117,118]. An RCT in older women found a multimodal exercise program resulted in neurocognitive improvements and increased levels of plasma BDNF[117], believed to be a peripheral biomarker of neurogenesis[119]. A 2 year multimodal intervention of diet, exercise, cognitive training and vascular risk monitoring found significant improvements in a comprehensive neuropsychological battery[120].

1.3.2.3 Hearing Loss

In Australia, hearing loss occurs in 49% of individuals over 75 years of age[121] and is associated with approximately 9% of the associated risk for dementia in mid-life[13]. Results from cohort studies have consistently shown that even mild levels of hearing loss have the potential to increase lifetime risk of dementia[122-126]. Although the underlying mechanism connecting hearing loss with cognitive decline is unclear, research has suggested several possible mechanisms. Hearing loss may contribute to cognitive load in vulnerable individuals leading to neuropathological change[127] or lead to social disengagement[128] or accelerated cerebral atrophy[129].

1.3.2.4 Depression

Depression is suggested to be both a risk factor and a symptom of AD, leading to considerable debate as to the causal direction of influence. Depression may represent an independent risk factor or a prodromal symptom of this disease, as the current research supports both hypotheses. Longitudinal cohort studies[130-132] display an association between the number of depressive episodes and dementia risk. However, one study[133]

followed participants for up to 28 years and found that depressive symptoms emerged only 10 years prior to dementia onset. Symptoms were higher in those who went on to develop dementia than those who did not; suggesting late-life depression had a greater impact on dementia risk than mid-life.

Dementia is known to affect stress hormones, neuronal growth factors and hippocampal volume[134], providing biological support for a dementia-AD association. Excessive secretion of stress related hormones reduce the production of neurotrophic factors, such as BDNF, inhibiting neurogenesis and potentially increasing an individual's vulnerability to amyloid deposition and vascular degeneration. Biological changes during depression can lead to brain volume reduction in the subgenual prefrontral, orbitofrontal and dorsolateral cortex of depressed individuals[135]. Evidence has also indicated a reduction in Aβ production through use of antidepressant prescriptions[136,137], such as citalopram[138]. Research has also illuminated sex differences in depression, with reductions in hippocampal volume reported in depressed men[139] and women experiencing extended durations of depression[135]. A systematic review into the sex-differences of depression as a risk factor for AD failed to find any meaningful difference; however the authors note this is likely due to methodological inconsistencies among the reported data[140].

Without a causal model research in this area remains unclear, this effect may be due to a period of heightened vulnerability or an early symptom of dementia. Depression may be both a risk factor and a prodromal symptom of AD[134].

1.3.2.5 Diet

Evidence from epidemiological, observational and interventional studies suggests an association between diet and AD. Accumulating evidence from original research,

systematic review[141] and meta-analysis[142] suggests dietary and nutritional factors influence the risk of developing AD.

Epidemiological findings are consistent regarding a diet characterised by higher intake of fruit, vegetables, cereals and legumes and lower intake of meat, high-fat dairies and sweets are associated with a reduced risk for AD [143-147]. Prospective research has also suggested that increased adherence to a Mediterranean diet (MeDi)[148], low to moderate alcohol consumption[149,150], lower carbohydrate consumption[151] and increased vitamin intake[152] are associated with a reduction in AD risk.

Higher intake of vitamins C and E[153], flavonoids[154], unsaturated fatty acids[94], fish[155], vitamin B12[156] and folate[157] have been associated to a reduction in AD risk. Individuals who adhere to a MeDi-styled diet (high intake of fruit, vegetables and fish; low intake of meat and dairy) display fewer vascular risk factors and reduced insulin concentrations, insulin resistance, oxidative stress and inflammation[158]. In a tri-factor model including physical activity, increased MeDi adherence was also associated with lower Aβ burden[159]. Recent research has alluded associations between a MeDi-type pattern and slower cognitive decline[147], reduction in risk of conversion from MCI to AD[158] and reduced mortality [144,160].

Due to consistency in research findings, several researchers have proposed models of causation underlying the observed diet-AD relations. Seneff*, et al.* [151] hypothesized the combined effect of excess dietary carbohydrates and deficient relative consumption of fats and cholesterols may contribute to disease risk (Figure 17). Cholesterol deficient neurons have significantly impaired function, leading to impaired glutamate signalling, mitochondrial and/or lysosomal dysfunction, increased risk of microbial infection and apoptosis[151]. Other researchers have also noted how elevated cholesterol can result in extracellular acidification, leading to an increase in the expression of tumor necrosis

factor alpha (TNF-α)[161]. TNF-α is a cytokine involved in neuroinflammation in initiating and propagating an inflammatory response and therefore may play a role in the pathological cascade that leads to AD[162]. Pasinetti, *et al.* [163] proposed a diet-AD model that incorporated high caloric intake and increased incidence of AD, however this evidence was based on mice models and not substantiated in humans.

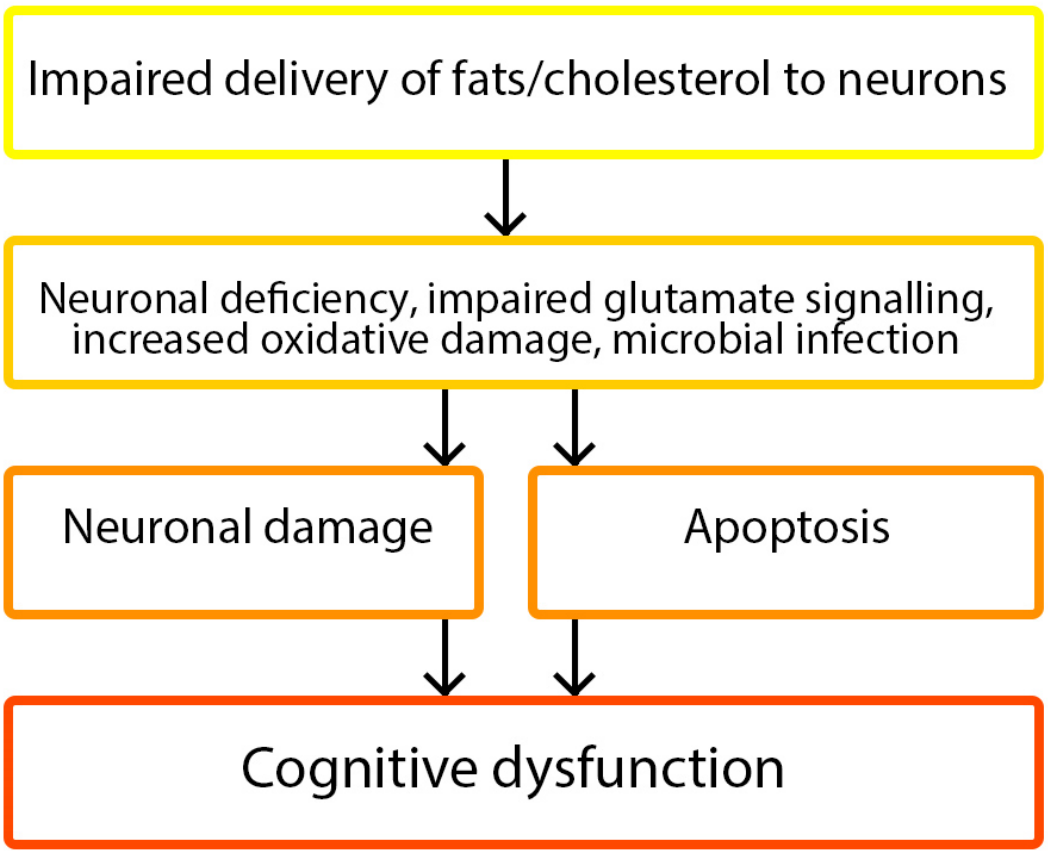

Figure 17: Potential diet-AD causal pathway highlighting excess carbohydrate intake as a possible mechanism underlying diet-AD associations. Created by E.Hill, modified from Seneff, *et al.* [151]

In order to reduce the global sociological, economic and healthcare burden due to dementia, research is warranted into the risk factors that may ameliorate AD symptomatology. With evidence for non-pharmacological impact upon the AD continuum, the impetus to examine potentially modifiable risk factors for AD is clear.

2 LITERATURE REVIEW

This chapter presents a literature review of the relationship between diet and Alzheimer's disease. The association between diet and clinical manifestations of Alzheimer's disease epidemiology (incidence and prevalence) will be collated and summarised. Furthermore, a systematic review and meta-analysis examining the association between diet and biomarkers of Alzheimer's disease will be presented.

This chapter contains a modified version of the following publication[164]:

Hill E, Goodwill AM, Gorelik A, Szoeke C. Diet and biomarkers of Alzheimer's disease: a systematic review and meta-analysis. Neurobiology of Aging. 2019; April: 45-52.

2.1 The association between diet & Alzheimer's disease

2.1.1 Diet and the incidence/prevalence of Alzheimer's disease

Evidence from several studies since the 1990s indicates a strong link between dietary patterns and AD incidence/prevalence[165-167]. However, several scholars have noted the substantial heterogeneity in dietary findings and their relationship to heterogeneity in research methodology. A systematic review by Loef*, et al.* [165] found zinc deficiency in AD subjects; however, was inconclusive as to whether zinc supplementation exerted a protective effect. Similarly, a review conducted by Kivipelto*, et al.* [167] suggested hypocholesteraemia was an additional risk factor for AD; however, other epidemiological evidence suggests the relationship fluctuates between different populations. A review by Luchsinger*, et al.* [168] found considerable inconsistencies with respect to the association between diet and AD, noting the lack of randomised trials to validate claims suggested in observational epidemiological research. A systematic review conducted by Yusufov*, et al.* [141] found 50 out of 64 studies found an association between diet and AD (Table 2).

In previous research, the majority of studies investigating diet-AD relations have evaluated AD using clinical diagnostic criteria. Within the 64 studies identified by Yusufov*, et al.* [141] there was substantial heterogeneity in both the dietary measure and the AD diagnostic criteria. Out of the 64 studies, a total of 141 unique dietary models were identified and several different methods of evaluating and diagnosing were utilised: 39 used the NINCDS-ADRDA criteria, 13 DSM-III, 6 DSM-IV, 6 Mini Mental Status Examination (MMSE), and 1 ICD-9. Despite considerable heterogeneity in methodology, Yusufov*, et al.* [141] found 50 out of 64 studies (78.1%) revealed a significant diet-AD relationship. Whether they investigated macronutrients,

antioxidants, beverages or the MD, the majority of studies supported the role of diet in clinical symptoms of AD (Table 2); however, only 10 of the 64 studies (16%) utilised neuroimaging to suggest an AD diagnosis and only six studies found by Yusufov*, et al.* [141] evaluated AD utilising biomarkers.

Study	Design	Setting	N	Age	Diet	Evaluation of AD	Significant diet-AD relationship reported?
Barberger-Gateau, *et al.* [169]	Prospective cohort	France	1674	68	Fish/meat	DSM-III	Yes
Barnerger-Gateau, *et al.* [170]	Prospective cohort	France	8085	65	Fish, meat, raw fruits, raw vegetable, cooked fruits or vegetables, wine, butter, goose/duck fat, corn oil, olive oil, colza oil, walnut oil, omega-3 rich oils, peanut oil, sunflower or grapeseed oil	DSM-IV	Yes
Berti, *et al.* [146]	Cross-sectional	USA	52	54	Five nutrient patterns (NP1, NP2, NP3, NP4, NP5)	Biomarkers	Yes
Commenges, *et al.* [154]	Cohort	France	1367	65	Flavonoids, vitamin C, wine	DSM-III, MMSE	Yes
Corrada, *et al.* [171]	Cohort	USA	579	70	Carotenoids, folate, vitamin C, vitamin E, vitamin B_6, vitamin B_{12}	NINCDS-ADRDA	Yes
Dai, *et al.* [172]	Prospective	USA	1836	72	Fruit and vegetable juice, tea drinking, wine (sake) drinking	DSM-IV, NINCDS-ADRDA	Yes
Daiello, *et al.* [173]	Retrospective cohort	USA	819	75	Fish oil	ADAS-cog, MMSE, biomarkers	Yes
Devore, *et al.* [174]	Cohort study	Netherlands	5395	68	High fish intake	NINCDS-ADRDA	No

Devore, *et al.* [175]	Prospective cohort	Netherlands	5395	68	Beta-carotene, flavonoids, vitamin C, vitamin E	NINCDS-ADRDA	Yes
Devore, *et al.* [176]	Population cohort	Netherlands	5395	66	Antioxidants	DSM-III, NINCDS-ADRDA	Yes
Douaud, *et al.* [177]	RCT	England	156	77	B vitamins	Biomarkers	Yes
Ikeda, *et al.* [178]	Prospective cohort	Netherlands	5395	68	Antioxidants, vitamin C, vitamin E	DSM-III, NINCDS-ADRDA	Yes
Ikeda, *et al.* [178]	Prospective cohort	Netherlands	5395	68	High cholesterol, high saturated fats, high total fats, high trans fats, low MUFA, low PUFA	NINCDS-ADRDA	No
Eskelinen, *et al.* [179]	Population-based cohort	Finland	1409	71	Coffee	NINCDS-ADRDA	Yes
Feart, *et al.* [147]	Prospective cohort	France	1410	76	Mediterranean diet	MMSE; NPE	No
Gardener, *et al.* [148]	Cross-sectional	Australia	1112	72	Mediterranean diet	MMSE; NPE	Yes
Gelber, *et al.* [180]	Nested case-control	USA	3494	52	Coffee	NINCDS-ADRDA, Autopsy lesions	Yes
Gelber, *et al.* [181]	Nested case-control	USA	3468	52	Diet score (healthiest)	NINCDS-ADRDA	No

Gray, *et al.* [182]	Prospective cohort	USA	2969	76	Vitamin C, Vitamin E, combined	NINCDS-ADRDA	No
Gu, *et al.* [183]	Prospective cohort	USA	1219	77	Mediterranean diet	DSM-III	Yes
Gu, *et al.* [184]	Prospective cohort	USA	2148	77	-	DSM-III	Yes
Gustaw-Rothenberg [185]	Cross-sectional	Poland	71	-	-	-	Yes
Huang, *et al.* [186]	Prospective	USA	5201	72	-	DSM-IV, NINCDS-ADRDA	Yes
Hughes, *et al.* [187]	Population-based cohort	Sweden	3779	84	Fruit and vegetable	DSM-IV, NINCDS-ADRDA	Yes
Kalmijn, *et al.* [188]	Prospective cohort	Netherlands	5386	68	Trans fat, saturated fat, cholesterol, total fat	NINCDS-ADRDA, MMSE, NPE	Yes
Kesse-Guyot, *et al.* [189]	Cross-sectional	France	3083	52	Mediterranean diet	-	No
Kim, *et al.* [190]	Prospective	South Korea	518	73	Folate, homocysteine, vitamin B_{12}	NINCDS-ADRDA	Yes
Laitinen, *et al.* [191]	Longitudinal	Finland	1449	50	PUFA, SFA	NINCDS-ADRDA, DSM-IV	Yes

Laurin, *et al.* [192]	Prospective	USA	2459	52.4	Beta-carotene, flavonoids, vitamin C, vitamin E	DSM-III, NINCDS-ADRDA	Yes
Li, *et al.* [193]	Population-based cohort	USA	2141	74.9	Cholesterol, high-density lipoprotein	NINCDS-ADRDA	No
Lindsay, *et al.* [194]	Prospective	Canada	4615	74.4	Coffee, wine	3MS, DSM-IV, NINCDS-ADRDA	Yes
Littlejohns, *et al.* [195]	Prospective	USA	1658	73.6	Vitamin D	NINCDS-ADRDA	Yes
Luchsinger, *et al.* [196]	Prospective cohort	USA	980	75.3	Vitamin C, vitamin E	NINCDS-ADRDA	No
Luchsinger, *et al.* [197]	Cohort	USA	980	75.3	Alcohol, beer, liquor, wine	DSM-IV, NINCDS-ADRDA	Yes
Luchsinger, *et al.* [198]	Longitudinal cohort	USA	965	75.8	Folate, vitamin B_{12}, vitamin B_6	NINCDS-ADRDA	Yes
Maia, *et al.* [199]	Case control	Portugal	54	70.8	Coffee	NINCDS-ADRDA	Yes
Mangialasche, *et al.* [200]	Cohort	Sweden	232	85.4	Vitamin E	NINCDS-ADRDA	Yes

Mielke, *et al.* [201]	Cohort	Sweden	1462	38-60	Cholesterol	NINCDS-ADRDA	No
Matthews, *et al.* [159]	Cross sectional	USA	45	54.0	Mediterranean diet	Biomarkers	Yes
Morris, *et al.* [202]	Prospective	USA	633	65.0	Vitamin C, Vitamin E	NINCDS-ADRDA	Yes
Morris, *et al.* [203]	Prospective	USA	815	73.3	Vitamin E, Vitamin C, Beta-carotene	NINCDS-ADRDA	Yes
Morris, *et al.* [204]	Longitudinal	USA	815	73.1	Saturated fat, trans fat	NINCDS-ADRDA	Yes
Morris, *et al.* [155]	Prospective	USA	815	73.1	Fish, omega-3 fatty acids	NINCDS-ADRDA	Yes
Morris, *et al.* [205]	Prospective cohort	USA	815	65.0	Niacin	NINCDS-ADRDA	Yes
Morris, *et al.* [207]	Prospective cohort	USA	1041	65.0	Folate, vitamin B_{12}, vitamin B_6	NINCDS-ADRDA	Yes
Morris, *et al.* [208]	Prospective cohort	USA	923	81.2	MIND	-	Yes

Mosconi, *et al.* [209]	Cross sectional	USA	52	54.0	Mediterranean diet	Biomarkers	Yes
Mosconi, *et al.* [210]	Cross sectional	USA	49	54.0	Folate, beta-carotene, vitamin B_{12}, vitamin D, saturated fats	Biomarkers	Yes
Mukamal, *et al.* [211]	Nested case control	USA	746	77.7	Alcohol	NINCDS-ADRDA	Yes
Ozawa, *et al.* [212]	Prospective cohort	Japan	1081	69.1	Calcium, magnesium, potassium	NINCDS-ADRDA	No
Ravaglia, *et al.* [213]	Cohort	Italy	816	74.0	Homocysteine, low folate	NINCDS-ADRDA	Yes
Rogers, *et al.* [214]	Matched case control	USA	46	73.4	Aluminium	NINCDS-ADRDA	Yes
Ruitenberg, *et al.* [215]	Prospective population based	Netherlands	7983	67.4	Alcohol	NINCDS-ADRDA	Yes
Scarmeas, *et al.* [144]	Case control	USA	1984	76.3	Mediterranean diet	DSM-III	Yes
Scarmeas, *et al.* [143]	Prospective cohort	USA	192	82.9	Mediterranean diet	DSM-III	Yes

Wu, *et al.* [216]	Prospective cohort	USA	2258	77.2	Mediterranean diet	DSM-III	Yes
Scarmeas, *et al.* [217]	Prospective cohort	USA	282	77.2	Mediterranean diet	DSM-III	Yes
Scarmeas, *et al.* [218]	Cross sectional	USA	282	80.3	Mediterranean diet	DSM-III	Yes
Seshadri, *et al.* [219]	Prospective	USA	1092	76.0	Homocysteine	NINCDS-ADRDA	Yes
Solomon, *et al.* [220]	Retrospective cohort	USA	9844	69.4	Cholesterol	ICD-9 code 331.0	Yes
Toro, *et al.* [221]	Prospective	Germany	500	62.4	Cholesterol	NINCDS-ADRDA	Yes
Wang, *et al.* [222]	Longitudinal	Sweden	370	75+	Folate, fruit intake, vitamin B_{12}	DSM-III	Yes
Williams [223]	Prospective cohort	USA	175	59.1	Fruit intake	AD mortality	Yes
Zandi, *et al.* [224]	Cross-sectional; prospective	USA	5092	75.2	Vitamin C, vitamin E, multivitamins, B-complex vitamins	DSM-III, MMSE	Yes

Table 2: Summary of studies investigating diet-AD relations. Modified from Yusufov, *et al.* [141]

2.1.2 Diet and biomarkers of Alzheimer's disease: a systematic review and meta-analysis

2.1.2.1 Introduction

Dementia incidence exponentially increases with age, doubling approximately every 5 years [225]. Alzheimer's disease (AD) is the leading cause of dementia, accounting for 60-70% of all cases [226] and is a global public health priority. The *Alzheimer's Disease International* World Alzheimer Report 2015 estimated 46.8 million people living with dementia worldwide and this number is expected to reach 131.5 million by 2050 [1]. Current pharmacological approaches, including cholinesterase inhibitors and NMDA receptor antagonists, are focused on treating AD symptomatology and are lacking therapeutic efficacy. Clinical trials that focus on reducing the pathological burden of AD biomarkers are failing [227]. Given the resistance to treatment, a growing body of research is now focusing on the preventative potential of modifiable risk factors in the AD pathology prior to the emergence of clinical symptoms.

Non-modifiable risk factors for AD include family history, ageing, head injury and carrying the epsilon 4 allele of the apolipoprotein E gene (APOE-ε4). The *2017 Lancet Commission on Dementia Prevention, Intervention and Care* found that approximately 35% of dementia is attributable to potentially modifiable lifestyle risk factors [13]. Epidemiological evidence indicates that up to 3 million AD cases worldwide could be prevented with a 10-25% reduction in known modifiable mid-life risk factors [98].

Diet and nutrition may offer potential for non-pharmacological prevention in AD. Epidemiological findings are consistent in showing that high adherence to dietary patterns characterized by high intake of fruit, vegetables, cereals and legumes and low intake of meat, high-fat dairies and sweets are consistently associated with a lower risk

of AD [143,145,147,148,228]. Prospective studies have also suggested that low to moderate alcohol consumption [150], lower carbohydrate consumption [151] and increased vitamin intake [152] are associated with a decreased risk for AD. In Australia, increased adherence to a Mediterranean diet (MeDi) has been associated with changes in Mini-Mental State Examination over 18 months [148] and a reduction in risk for cognitive decline (Gardener et al., 2015). An ecological study found AD prevalence in Japan was associated with a prolonged nutritional transition from the traditional Japanese diet to the Western diet, where AD rates rose from 1% in 1985 to 7% in 2008 [229].

In AD, the onset of clinical symptoms is preceded by a preclinical prodromal phase of neurochemical, neuropathological, functional and structural brain changes [19,230]. Therefore the identification of presymptomatic divergence from normal brain ageing is important as this may serve as a marker for early detection of people that may benefit from preventative intervention strategies. Central to the AD pathogenesis is the proliferation, aggregation and deposition of two proteins in the brain: beta-amyloid (Aβ) and the microtubule associated protein tau [231]. Biomarkers provide unique insights into the underlying neuropathology of AD, at times operating independently of the clinical and neuropsychological manifestations.

In recent years, the use of positron emission tomography (PET) scans for the *in vivo* measurement of cerebral AD biomarkers has been increasingly utilised and the validity of Aβ PET radiotracers for the diagnosis of AD has been documented [38]. Increased amyloid-PET positivity and low cerebrospinal fluid Aβ have been shown to precede clinical AD manifestations by many years [19]; however, neuroimaging lacks cost-effectiveness and (cerebrospinal fluid) CSF extraction is invasive. A robust inverse relationship has been observed between cortical Aβ binding and levels of CSF Aβ42 in cognitively normal individuals [49]. Research has suggested that central and peripheral pools of Aβ may be in a state of equilibrium and that the changes in blood levels of

Aβ42 may be reflected in cerebral Aβ deposition [232]. More recently, the utility of plasma to detect AD pathophysiology has been clinically validated in several cohort studies [51,233,234]. The stability and sensitivity of plasma Aβ measurements has also been reported to suggest a blood-brain transportation mechanism of Aβ [235]. In the NIA-AA Research Framework [20], blood-based biomarkers were regarded as showing promise as a future screening tool for AD given they are less invasive and expensive than existing cerebral and CSF biomarkers.

Summaries of the existing evidence reveal an association between healthy diet adherence and reduced AD incidence and prevalence. A recent systematic review found 50 out of 64 studies revealed a significant association between diet and AD incidence [236]; however, no review has investigated this relationship with respect to the hallmark AD biomarkers that manifest decades prior to clinical symptomatology. The aims of this systematic review were to summarize the evidence relating diet and nutrition to the hallmark AD biomarkers (tau and beta-amyloid), identify methodological constraints and provide future research directions.

2.1.2.2 Materials and methods

We searched published scientific literature for prospective cohort studies and randomized controlled trials of adult human participants reporting diet/nutritional intake and AD biomarkers. This systematic review adhered to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement guidelines [237] (Table 3). The protocol was designed a priori and was registered with PROSPERO (CRD42017076389).

	Section/topic	Checklist item	Reported
	TITLE		
1	Title	Identify the report as a systematic review, meta-analysis, or both.	Yes
	ABSTRACT		
2	Structured summary	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Yes
	INTRODUCTION		
3	Rationale	Describe the rationale for the review in the context of what is already known.	Yes
4	Objectives	Provide an explicit statement of questions being addressed with reference to PICOS.	Yes
	METHODS		
5	Protocol and registration	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Yes
6	Eligibility criteria	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Yes
7	Information sources	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Yes
8	Search	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Yes
9	Study selection	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Yes
10	Data collection process	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Yes
11	Data items	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Yes

12	Risk of bias in individual studies	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Yes
13	Summary measures	State the principal summary measures (e.g., risk ratio, difference in means).	Yes
14	Synthesis of results	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	Yes
15	Risk of bias across studies	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Yes
16	Additional analyses	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Yes
	RESULTS		
17	Study selection	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Yes
18	Study characteristics	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Yes
19	Risk of bias within studies	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Yes
20	Results of individual studies	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Yes
21	Synthesis of results	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Yes
22	Risk of bias across studies	Present results of any assessment of risk of bias across studies (see Item 15).	Yes
23	Additional analysis	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Yes
	DISCUSSION		

24	Summary of evidence	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Yes
25	Limitations	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Yes
26	Conclusions	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Yes
	FUNDING		
27	Funding	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Yes

Table 3: PRISMA Checklist for Systematic Reviews and Meta Analyses. From Moher, *et al.* [237]

2.1.2.2.1 Search strategy and article selection

Searches were conducted in MEDLINE, PubMed, PsycINFO, Google Scholar and SCOPUS databases to identify peer-reviewed articles that examined the relationship between diet and/or nutrition and AD biomarkers in the last two decades (1st January 1997-12th September 2017). Articles were extracted, quality assessed and double blind screened by two authors (EH/AG) and were included if they reported on associations between diet/nutrition and physiological AD biomarkers. The National Institutes of Health (NIH) quality assessment tool was used to assess bias within included studies (Table 4). For all four databases, the exact search date was from 1st January 1997 through 12th September 2017 and updated thereafter. Google Scholar searches were performed to identify additional articles.

Depending on the mesh headings, keywords and database requirements, a Boolean search strategy was conducted with the general following logic: (“diet” OR “dietary patterns” OR “dietary factors” OR “food” OR “nutrition”) AND ("tau" OR “p-tau” OR “t-tau” OR “Aβ42” OR “Aβ40” OR “Aβ” OR “βA” OR "amyloid” OR "β amyloid OR "β-amyloid" OR "amyloid-β" OR "amyloid β") NOT (rat OR rats OR mice OR mouse OR cell OR cells) OR AB(rat OR rats OR mice OR mouse OR cell OR cells). Studies were included in the review if they (1) were original studies; (2) contained multiple subjects (no case reports); (3) included human subjects only; (4) utilized a self-reported dietary/nutritional measure (no pathological nutritional marker) and (5) examined the AD biomarker (tau/amyloid) in cerebral, CSF or plasma levels. No limitations were set on study cohort, cohort classification (cognitively normal [NL]/AD/mild cognitive impairment [MCI]), country or setting. Cross-sectional, longitudinal and interventional studies were included.

	Quality Assessment of Controlled Intervention Studies	Freund-Levi, *et al.* [238]	Bayer-Carter, *et al.* [239]	Baker, *et al.* [240]	Hanson, *et al.* [241]	Freund Levi, *et al.* [242]	Chen, *et al.* [243]	Miller, *et al.* [244]
1	Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?	Y	Y	Y	Y	Y	Y	Y
2	Was the method of randomization adequate (i.e., use of randomly generated assignment)?	Y	Y	Y	NR	NR	Y	Y
3	Was the treatment allocation concealed (so that assignments could not be predicted)?	Y	Y	Y	Y	Y	Y	Y
4	Were study participants and providers blinded to treatment group assignment?	Y	Y	Y	Y	Y	N	Y
5	Were the people assessing the outcomes blinded to the participants' group assignments?	Y	Y	Y	Y	Y	N	Y
6	Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)?	Y	Y	Y	Y	NR	Y	Y
7	Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment?	Y	Y	Y	Y	Y	N	Y
8	Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower?	Y	Y	NR	Y	Y	Y	Y
9	Was there high adherence to the intervention protocols for each treatment group?	Y	Y	Y	Y	Y	Y	Y
10	Were other interventions avoided or similar in the groups (e.g., similar background treatments)?	Y	Y	Y	Y	Y	Y	Y

11	Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	Y	Y	Y	Y	Y	Y	N
12	Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power?	NR	NR	NR	NR	NR	Y	NR
13	Were outcomes reported or subgroups analyzed prespecified (i.e., identified before analyses were conducted)?	Y	Y	Y	Y	Y	Y	Y
14	Were all randomized participants analyzed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis?	Y	Y	Y	Y	Y	Y	Y

Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies		Gu, *et al.* [245]	Matthews, *et al.* [159]	Mosconi, *et al.* [209]	Berti, *et al.* [246]	Merrill, *et al.* [247]	Taylor, *et al.* [248]	Hill, *et al.* [249]	Berti, *et al.* [250]
1	Was the research question or objective in this paper clearly stated?	Y	Y	Y	Y	Y	Y	Y	Y
2	Was the study population clearly specified and defined?	Y	Y	Y	Y	Y	Y	Y	Y
3	Was the participation rate of eligible persons at least 50%?	Y	Y	Y	Y	Y	Y	Y	Y
4	Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Y	Y	Y	Y	Y	Y	Y	Y

5	Was a sample size justification, power description, or variance and effect estimates provided?	Y	Y	Y	Y	N	Y	Y	Y
6	For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Y	CD	CD	CD	CD	N	CD	Y
7	Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Y	Y	Y	Y	Y	Y	Y	Y
8	For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Y	Y	Y	Y	Y	Y	Y	Y
9	Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Y	Y	Y	Y	Y	Y	Y	Y
10	Was the exposure(s) assessed more than once over time?	Y	N	N	N	CD	CD	Y	Y
11	Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Y	Y	Y	Y	Y	Y	Y	Y
12	Were the outcome assessors blinded to the exposure status of participants?	N	N	N	N	Y	CD	N	N
13	Was loss to follow-up after baseline 20% or less?	Y	NA	NA	NA	NA	NA	Y	Y
14	Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Y	Y	Y	Y	Y	Y	Y	Y

Table 4: NIH Study Quality Assessment Tool for studies included in the systematic review and meta-analysis

2.1.2.2.2 Data Extraction

Data extraction involved the first author extracting the following characteristics from the articles: lead author, year of publication, follow-up period, country/setting, sample size, sex, mean age (and standard deviation) in years, cohort name, cohort classification, diet/nutrient measure, evaluation of AD biomarker, covariates, statistical methodology, effect size, standardized beta coefficients (where available) and standard error (where available). To clarify variables that may have influenced outcomes, subgroup analysis was performed by grouping the data according to cohort classification (NL vs. MCI vs. AD), dietary measure (Glycemic vs. MeDi vs. Nutrients vs. Omega-3 vs. Folic vs. Vit D), AD biomarker (tau vs. Aβ) and *in vivo* method of AD biomarker procurement (cerebral vs. CSF vs. plasma).

2.1.2.2.3 Quantitative analysis

For the quantitative analysis, where populations were drawn from the same participant pool we excluded the earliest publication (Bayer-Carter et al., 2011; Freund-Levi et al., 2009), we excluded studies where global coefficient values were not reported (Berti et al., 2015; Berti et al., 2018; Matthews et al., 2014) and randomized control trials (RCTs) that administered dietary interventions that were not comparable to other studies (Baker et al., 2012; Chen et al., 2016; Freund-Levi et al., 2014; Hanson et al., 2013; Matthews et al., 2014; Miller et al., 2016). For studies using comparable dietary assessment methods, we extracted standardized beta-coefficients and error estimate data (where available) of dietary effects on AD biomarkers. Due to heterogeneity in the direction of the effect of diet on AD biomarkers, we reversed the effect size for one RCT (Taylor et al., 2017) that investigated a high glycemic load diet and sugar/carbohydrate intake on cerebral Aβ deposition. Studies that provided multiple nutritional or dietary relationships with both Aβ and tau were separated by AD

biomarker. Where studies did not provide estimates of error, standard error (SE) was estimated using a simulation model. For each cohort, baseline summary statistics and variable distribution were used to reproduce the study cohort by applying a probabilistic model. Regression models were then constructed following the methodology described in each study and at least 500 simulations were run to generate a reliable estimate of SEs. Meta-regression was then conducted in STATA on Windows operating system. Heterogeneity was assessed using Cochran's Q-test and I2-statistic.

2.1.2.3 Results

2.1.2.3.1 Study characteristics

2.1.2.3.1.1 Description of studies

A total of 2726 records were screened (Figure 18). 15 studies met the inclusion criterion for qualitative analysis (7 RCTs, 7 cross-sectional, 1 longitudinal) and 5 studies were included in the quantitative analysis (5 cross-sectional).

Table 5 provides sample and study characteristics for the 15 included studies. Eleven of the 15 studies (73.3%) were conducted in the United States, 2 in Sweden, 1 in China and 1 in Australia. Several studies recruited participants from larger population pools; however, as the reported cohorts were unique populations these studies were included in the systematic review. A total of 2068 (68% female) subjects were included in the studies, sample sizes ranged from 24 to 1,219 participants. 1698 of these were defined as cognitively normal and 370 subjects were defined as MCI, amnestic MCI (aMCI) or AD. Seven studies were RCTs with a dietary intervention range of 4 weeks to 6 months. Seven studies were cross-sectional and one longitudinal observational study was identified. Fourteen studies provided age in years with standard deviation and mean sample ages were 63.4 (SD = 8.2) years. Although heterogeneous, the identified studies were of modest methodological quality and adjusted for a similar battery of covariates (Table 4). The main sources of reduced quality of the included studies were non-

reporting of statistical power/variability, exposure-outcome order and multiple exposure measurements. I2 statistic was 99.65%.

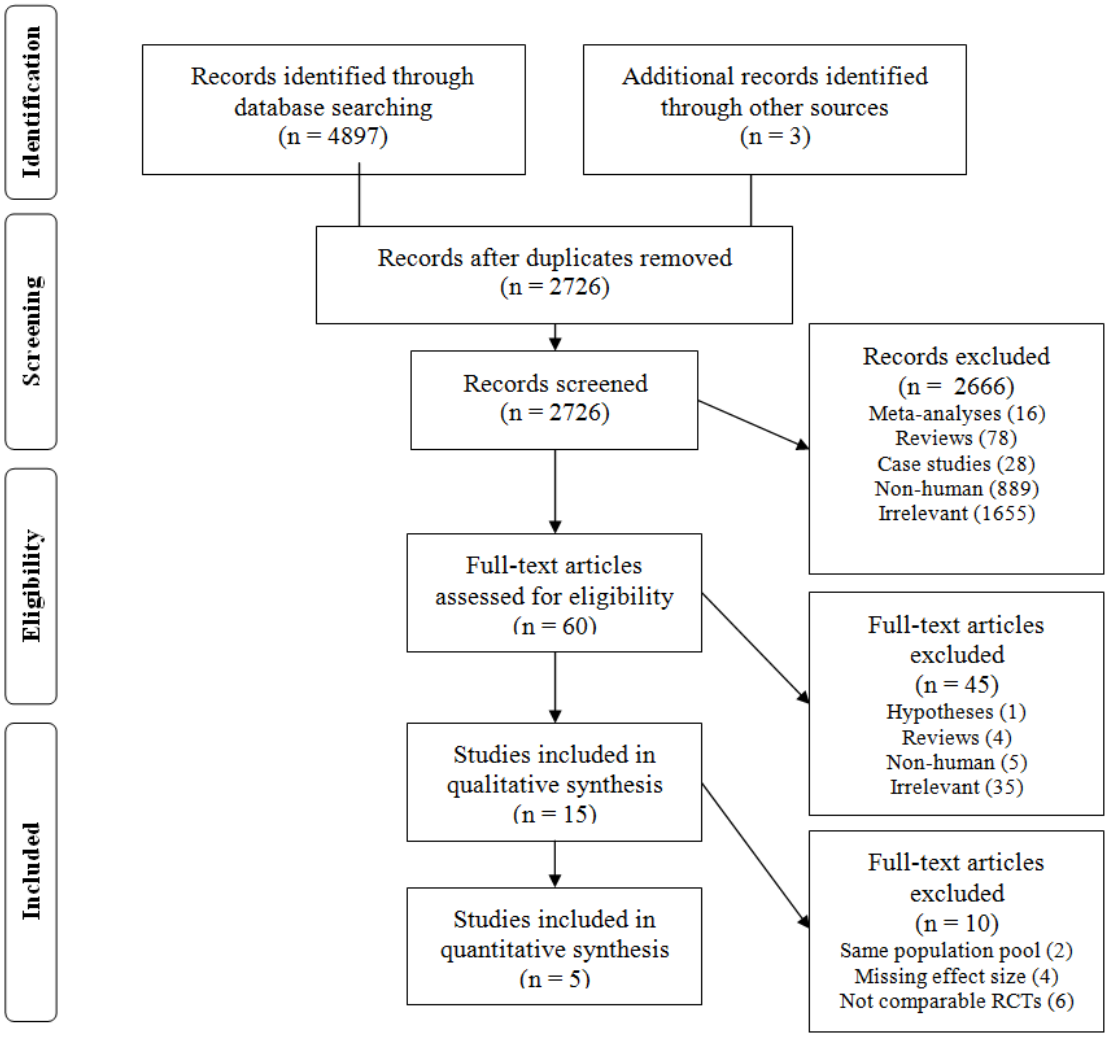

Figure 18: Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) diagram for systematic review of biomarkers of Alzheimer's disease and diet [237]

Study	Design (Follow-Up)	Country	N	Cohort	Age (years ± SD)	Measure (Diet/Nutrition)	AD Biomarker	Classification
Baker, *et al.* [240]	RCT (4 weeks)	USA	41	Veteran Affairs Puget Sound Health Care System	67 ± 7	Low saturated fat/low glycemic index or a high saturated fat/high glycemic index	CSF: Tau, Aβ42	Normal, MCI (MSE)
Bayer-Carter, *et al.* [239]	RCT (4 weeks)	USA	49	Veteran Affairs Puget Sound Health Care System	67 ± 7	Low saturated fat/low glycemic index or a high saturated fat/high glycemic index	CSF: Tau, Aβ40, Aβ42	Normal, aMCI
Berti, *et al.* [246]	XS	USA	52	New York University Langone School of Medicine	54 ± 12	Of 35 nutrients that have been associated with cognitive function and AD, 5 nutrient patterns (Harvard/Willet FFQ) identified using PCA	PET: Aβ (^{11}C PiB)	Normal
Freund Levi, *et al.* [242]	RCT (6 months)	Sweden	33	OmegAD	-	Supplementation of docosahexaenoic acid-rich omega-3 fatty acid preparation	CSF: P-tau, T-tau, Aβ42	AD
Freund-Levi, *et al.* [238]	RCT (6 months)	Sweden	35	OmegAD	70.3 ± 8.2	Supplementation of docosahexaenoic acid-rich omega-3 fatty acid preparation	CSF: P-tau, T-tau, Aβ42	AD
Hanson, *et al.* [241]	RCT (4 weeks)	USA	47	Veteran Affairs Medical Centre	NL:69 ± 7 aMCI:67 ± 6	Low saturated fat/low glycemic index or a high saturated fat/high glycemic index	CSF: Lipid deleted Aβ40 Aβ42	Normal, aMCI
Matthews, *et al.* [159]	XS	USA	45	New York University	54 ± 11	MeDi adherence (Harvard/Willett semi-	PET: Aβ (^{11}C PiB)	Normal

				Langone School of Medicine		quantitative FFQ) dichomitised into HIGH/LOW		
Merrill, *et al.* [247]	XS	USA	44	University of California	62.6 ± 10.7	MeDi adherence (5-point Likert scale) dichotimised into HIGH/LOW	PET: FDDNP (Aβ and tau)	MCI, subjective memory impairment
Mosconi, *et al.* [209]	XS	USA	49	New York University - Alzheimer's Disease Core Center	54 ± 11	10 AD related nutrients (Willett's semi-quantitative FFQ), alcohol, supplements	PET: Aβ (^{11}C PiB)	Normal
Gu, *et al.* [245]	XS	USA	1,219	Washington Heights/Hamilton Heights Columbia Aging Project	75.4 ± 6.1	10 nutrients (SFA, MUFA, ω-3 PUFA, ω-6 PUFA, vitamin E, vitamin C, β-carotene, vitamin B12, folate, and vitamin D)	Plasma: Aβ40 Aβ42	Normal
Chen, *et al.* [243]	RCT (6 months)	China	121	TFA-AD Trial, Huanhu Hospital, Tianjin	68 ± 8	Folic acid daily (1.25 mg.d) or placebo	Plasma: Aβ40 Aβ42 Aβ40/42	AD
Miller, *et al.* [244]	RCT (8 weeks)	USA	24	Phoenix (Volunteers recruited)	64.3 ± 10.9	Vitamin D weekly supplementation (50,000 IUs of cholecalciferol) or placebo	Plasma: Aβ40	Normal
Taylor, *et al.* [248]	XS	USA	128	Alzheimer's Prevention through Exercise (APEX)	71.3 ± 5.1	Sugar/carbohydrate intake and glycemic load (DHQ FFQ) HGLD Pattern (High glycemic load diet)	PET: Aβ (^{18}F Florbetapir)	Normal
Hill, *et al.* [249]	XS	Australia	111	Women's Healthy Ageing Project	70.0 ± 2.6	MeDi adherence (DQES v2) on 0-18 point scale	PET: Aβ (^{18}F Florbetaben)	Normal

Berti et al. (2018)	LT	USA	70	New York University Langone School of Medicine	MeDi-: 50 ± 9 MeDi+ 49 ± 9	MeDi adherence (Harvard FFQ) dichotomised into MeDi+/MeDi-	PET: Aβ (^{11}C PiB)	Normal

Table 5: Study characteristics of included studies

Thirteen studies reported a significant relationship. Of these; 4 studies found a higher glycemic load was related to increased AD biomarker burden in CSF [239-241] and the brain [248], 3 reported beneficial supplementation effects in CSF [238,242] & plasma [244] and 6 studies found adherence to a MeDi or 'AD-protective' (Berti et al., 2015) dietary pattern conferred a reduction in Aβ/tau burden or ratio [159,209,245-247,250]. 2 studies did not report a significant effect [238,249].

2.1.2.3.1.2 Dietary measures

The studies included in this systematic review generated a total of 11 unique dietary models. Of the 15 studies, 3 studies investigated the intake of nutrients that had been previously associated with AD or cognitive function [209,245,246]. Four studies measured glycemic indices and their relationship to Aβ40/42 in CSF [239-241] and the brain [248]. Four studies investigated adherence to the MeDi on a 2 [159,250], 5 [247] or 19 [249] point scale. Four studies investigated nutritional supplementation; omega-3[238,242], vitamin D [244] and folic acid [243].

2.1.2.3.1.3 AD biomarkers

Seven studies utilized positron emission tomography (PET) imaging (4 used ^{11}C Pittsburgh compound B [PiB] [159,209,246,250], 1 used ^{18}F Florbetapir [248], 1 used ^{18}F Florbetaben [249] and 1 used FDDNP [247]), 5 studies measured CSF levels [238-242] and 3 studies measured plasma biomarkers of AD [243-245]. All 15 studies investigated Aβ, while 5 investigated Aβ and tau [238-240,242,247] . Aβ was measured in CSF (Aβ40, Aβ42), plasma (Aβ40, Aβ 42 and Aβ40/42) and PET scans (^{11}C PiB, FDDNP and ^{18}F Florbetaben). Tau was measured in CSF (P-tau, T-tau) and PET scans (FDDNP binding).

2.1.2.3.2 Study findings

2.1.2.3.2.1 PET Imaging

Results are summarized in Table 6. Six out of the 7 studies that investigated PET imaging found a significant relationship between their dietary model and brain AD biomarker (Berti et al., 2015; Berti et al. 2018; Hill et al., 2018; Matthews et al., 2014; Merrill et al., 2016; Mosconi et al., 2014; Taylor et al., 2017) . Only one study utilised a non-NL cohort (Merrill et al., 2016). We identified four studies that utilized PiB = Pittsburgh Compound-B (PiB) PET imaging in a healthy (non MCI/AD) community cohort based in New York [159,209,246]. In their cross-sectional analysis, Berti*, et al.* [246] utilised factor analysis to define nutrient patterns that were associated with PiB retention, defining an AD protective nutrient pattern was consistent with higher intake of fresh fruit and vegetables, whole grains fish and low-fat dairies; and a lower intake of sweets, fried potatoes, high fat dairies, processed meat and butter. Several years later, Berti*, et al.* [250] reported on the only longitudinal study to date investigating diet and AD biomarkers, finding the MeDi- group showed significantly higher PiB-PET Aβ deposition in AD-affected regions at baseline than the MeDi+ group. Longitudinally, the MeDi- group displayed significant increases in Aβ in these regions that were greater than those in the MeDi+ group [250]. Matthews*, et al.* [159] dichotomised participants into high(+)/low(-) adherence to the MeDi, finding greater PiB retention in MeDi- compared with MeDi+. Mosconi*, et al.* [209] found higher intake of vitamin B_{12}, vitamin D and ω-3 polyunsaturated fatty acids (PUFA) were associated with lower Aβ load as detected by PiB. Two studies, one in Australia [249] and one in the United States [248], utilised ^{18}F radiotracers to investigate Aβ burden in elderly participants. Taylor*, et al.* [248] found a high glycemic diet was associated with higher global and regional Aβ burden as measured by ^{18}F florbetapir in cognitively normal participants. Independent glycemic measures such as sugar intake, carbohydrate intake and glycemic load, were also

associated with global and regional Aβ burden. Hill*, et al.* [249] found no association between MeDi adherence and Aβ burden as measured by ^{18}F florbetaben in both APOE-ε4 +/- healthy female participants. One study utilised FDDNP to measure the relationship between self-reported adherence to a MeDi dietary pattern and Aβ burden in participants diagnosed with MCI or subjective memory impairment (SMI) [247]. Regardless of cognitive status (MCI/SMI), a MeDi-type dietary pattern was associated with lower FDDNP-PET Aβ binding in AD associated regions such as the frontal, parietal, medial and lateral temporal, posterior cingulate [247].

Dietary Pattern	Number of Studies	Cohort Classification	AD Biomarker	Design	Significant Results Reported?	Significant Y/N (%)	Direction of association
Glycemic	4	1 NL 2 NL & aMCI 1 NL & MCI	1 CSF Aβ, 2 CSFAβ & Tau, 1 PET Aβ	3 RCTs, 1 XS	Yes	4/0 (100%)	Low glycemic diet increased Aβ in CSF. High glycemic diet associated with increased cerebral Aβ burden.
MeDi	4	3 NL 1 MCI	4 PET Aβ	3 XS, 1 LT	Yes	3/4 (75%)	Adherence to MeDi associated with reduction in cerebral Aβ burden.
Nutrients	3	3 NL	2 PET Aβ, 1 plasma Aβ	3 XS	Yes	3/3 (100%)	Higher intake of vitamin B12, vitamin D, ω-3 PUFA associated with lower cerebral Aβ burden. Lower cerebral Aβ burden associated with higher intakes of fresh fruit and vegetables, whole grains fish and low-fat dairies; and a lower intake of sweets, fried potatoes, high fat dairies, processed meat and butter.
Omega-3	2	2 AD	2 CSF Aβ & Tau	2 RCTs	Yes	1/2 (50%)	Supplementation of docosahexaenoic acid associated with decrease in CSF tau.
Folic	1	1 AD	1 plasma Aβ	1 RCT	Yes	1/1 (100%)	Supplementation of folic acid decreased plasma Aβ
Vit D	1	1 NL	1 plasma Aβ	1 RCT	Yes	1/1 (100%)	Supplementation of vitamin D increased plasma Aβ in vitamin D insufficient older adults.

Table 6: Summary of results grouped by dietary pattern

2.1.2.3.2.2 CSF Biomarkers

This systematic review found 4 out of the 5 included studies found a significant effect of diet/supplementation on CSF AD biomarkers. Two studies investigated AD cohorts (Freund-Levi et al., 2009; Freund Levi et al., 2009) and three studies investigated NL and MCI/aMCI (Baker et al., 2012; Bayer-Carter et al., 2011; Hanson et al., 2013). Four studies investigated either phosphorylated (P-) or total (T-) tau levels in CSF as well as Aβ [238-240,242] and 1 study investigated lipid deleted Aβ40 and Aβ42 [241]. Freund-Levi*, et al.* [238] found no significant effect of a docosahexaenoic acid (DHA) intervention on CSF biomarkers of AD (Aβ42, P-tau, T-tau); however, later reported a significant inverse correlation between levels of CSF P-tau/T-tau and levels of DHA [242]. Three studies investigated glycemic indices and AD biomarkers of participants in the Veteran Affairs Puget Sound Health Care System [239-241]. Bayer-Carter*, et al.* [239] found a low-saturated fat/low-glycemic index dietary intervention increased Aβ42 for amnestic MCI (aMCI) participants, yet decreased Aβ42 for the healthy adults. Conversely, the high-saturated fat/high-glycemic diet increased Aβ42 for healthy adults, yet had no significant effect on the aMCI participants [239]. In a later study drawn from the same participant pool, high levels of physical activity attenuated the effects of the high-saturated fat/high-glycemic diet on Aβ42 in healthy adults yet potentiated the effects of the low-saturated fat/low-glycemic index diet on MCI participants [240]. Hanson*, et al.* [241] followed up by investigating lipid-deleted Aβ40 and Aβ42, finding the high-saturated fat/high-glycemic diet significantly increased lipid-deleted Aβ42, whilst the low-saturated fat/low-glycemic diet tended to be associated with a decrease in Aβ40.

2.1.2.3.2.3 Plasma

Three studies investigated plasma biomarkers of AD and all of these described significant findings regarding dietary associations with plasma biomarkers of AD. Two studies investigated healthy NL participants (Gu et al., 2012; Miller et al., 2016) and one studied an AD cohort (Chen et al., 2016). Gu*, et al.* [245] investigated the cross sectional association of 10 nutrients with Aβ40 and Aβ42 in a large cohort (n = 1,219) of healthy adults, finding that higher intake of ω-3 PUFA was significantly associated with a reduction in Aβ40 (unadjusted) and Aβ42. The other two studies investigating plasma AD biomarkers were RCTs, one investigating a 6 month folic acid intervention in China [243], the other was based in the United States investigating vitamin D supplementation [244]. Given low folate levels have been associated with lower AD risk [251] and AD patients have low folate and vitamin B12, Chen*, et al.* [243] conducted an RCT on newly diagnosed AD patients being treated with donepezil. Participants in the intervention group displayed significantly higher MMSE means, a higher Aβ42/ Aβ40 ratio and lower Aβ40 than the control group [243]. In an 8-week trial in vitamin D insufficient adults, Miller*, et al.* [244] investigated whether vitamin D treatment (50,000 IU/week) had an impact on Aβ40 clearance. This study found vitamin D supplementation significantly enhanced brain Aβ transportation to the periphery. Miller*, et al.* [244] found a correlation between Aβ40 and total 25-hydroxyvitamin D in participants aged over 60 years, suggesting older individuals may have a stronger response to the treatment than their younger counterparts.

2.1.2.3.3 Quantitative analysis

Five studies were included in the meta-analysis (Gu et al., 2012; Hill et al., 2018; Merrill et al., 2016; Mosconi et al., 2014; Taylor et al., 2017). All five studies provided standardized effect sizes for the effect of diet on Aβ in either PET scans (Hill et al., 2018; Merrill et al., 2016; Mosconi et al., 2014; Taylor et al., 2017) or plasma measures

(Gu et al., 2012). One study provided effect sizes and SEs for tau in addition to Aβ (Merrill et al., 2016); however, this was not comparable to any other study therefore only the Aβ relationship is reported in the quantitative analysis. Pooled results suggest that dietary components are associated with a reduction in Aβ deposition. Based on 5 pooled studies with 19 effect sizes, the meta-analysis of Aβ deposition outcomes revealed a significant association between increased adherence to a MeDi or increased adherence to an 'AD-protective' dietary pattern and a 0.11 (95%CI 0.04-0.17, p=0.002) point lower deposition in Aβ. Results of the meta-analysis have been summarized in a forest plot (Figure 19) and funnel plot (Figure 20). Studies tended to be within 95% CI simulated estimates for SE, however one study (Gu *et al*, 2012) displayed a much larger standardized beta coefficient (ES -10.13, 95% CI -14.11, -6.15) compared to all other studies.

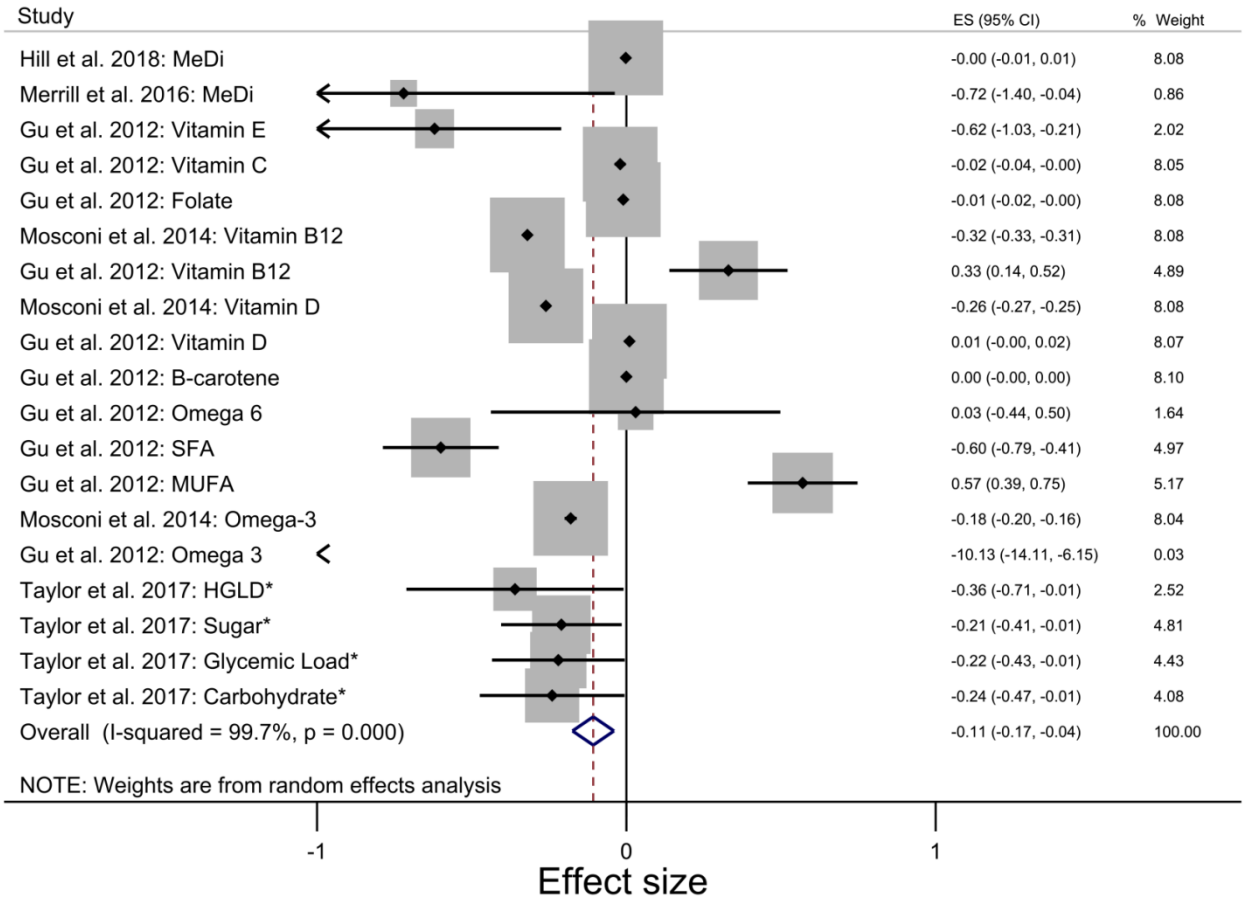

Figure 19: Forest plot for the standardized effect size (beta-coefficient) of diet on Aβ

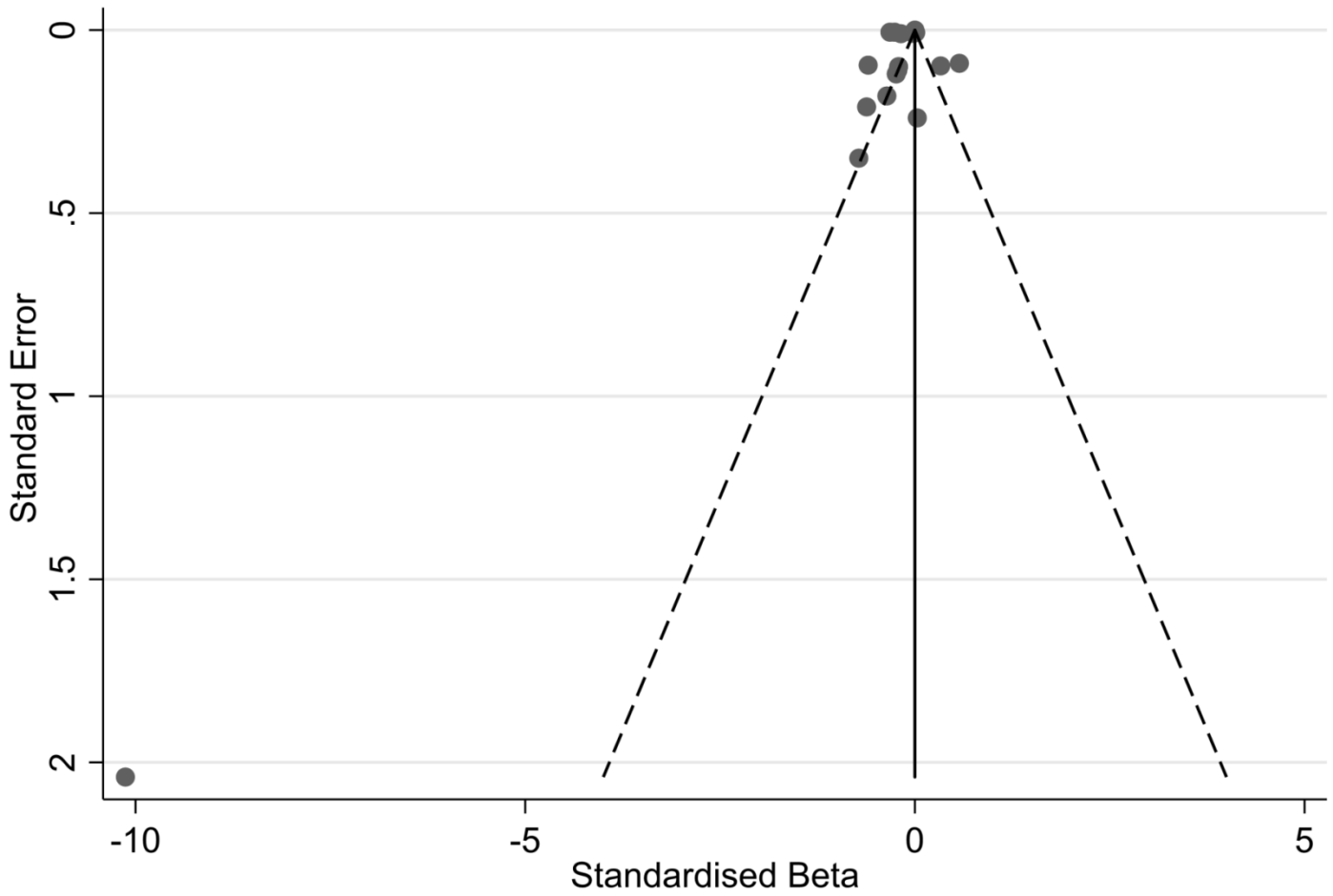

Figure 20: Funnel plot with pseudo 95% confidence interval estimates

2.1.2.4 Discussion

Given the existing evidence reporting a dietary effect on AD prevalence, this systematic review and meta-analysis supports the notion that diet and nutrition display potential for non-pharmacological prevention. Of the 15 studies that were identified during this systematic review of the evidence, 13 studies reported a statistically significant relationship, supporting the promising notion that diet may have preventative potential for AD. We identified substantial heterogeneity in dietary measures (11 unique models) and AD biomarkers (7 unique measures), although the studies adjusted for a similar battery of demographic, lifestyle and physical covariates. Supplementation of vitamin D/folic/ω-3 and adherence to a dietary pattern emphasizing a higher intake of fruit, vegetables, whole grains, oily fish and low-fat dairy was associated with a reduction in AD biomarker burden. Conversely, a diet characterised by consumption of high-glycemic, high saturated fat foods were associated with an increase in AD biomarker burden. Meta-analysis revealed a small but significant association between increased adherence to a MeDi or 'AD-protective' dietary pattern and lower AD biomarker burden. The findings of this review support the notion of diet as a potential modifiable risk factor for AD; however, the underlying mechanisms of dietary contributions to the mitigation of AD pathology remain a challenge for future research.

In cross-sectional (Matthews et al., 2014; Merrill et al., 2016) and longitudinal (Berti et al., 2018) research, adherence to a MeDi was associated with lower Aβ deposition. These data indicate that diet may influence AD progression and this review supports the notion of a possible pathophysiological relationship, as has been described in several clinical studies to date [147] [228]

Participants in the studies identified were predominantly cognitively normal; however, we did observe investigations along the disease spectrum (NL > MCI > AD). Subgroup

analysis identified that all AD measurements were investigated in at least one cognitive classification (NL, MCI or AD) and we observed no between-study difference in findings that investigated the same biomarker or method of measurement. However, we did observe within-study difference in one cohort consisting of multiple cognitive classifications. Bayer-Carter*, et al.* [239] reported an opposite association for NL/MCI individuals adhering to a HIGH\LOW fat/glycemic index dietary intervention. The LOW diet increased CSF Aβ42 concentrations for the aMCI group; however, increased CSF Aβ42 for the NL group. Bayer-Carter*, et al.* [239] speculate this relationship may be due to a tipping point of CSF concentrations, as have been described in animal models (DeMattos et al., 2002; Kawarabayashi et al., 2001).

We observed several RCTs (Freund-Levi et al., 2009; Freund Levi et al., 2014) and observational studies (Gu et al., 2012; Mosconi et al., 2014) reported a positive effect of ω-3 PUFA consumption on serum and CSF AD biomarker burden. Highly unsaturated fatty acids, such as ω-3 and ω-6, have been seen in lower levels in AD compared with controls [252]; however, we observed significant and non-significant effects on PUFA supplementation. This systematic review supports the notion that the beneficial effects of ω-3 PUFA consumption could be explained by a biomarker mechanism. PUFAs have been suggested to have neuroprotective and anti-inflammatory effects through Aβ clearance[253]; however, the relationship between ω-3 PUFA and AD biomarkers may be mediated by another factor, such as cognitive function [254], that were not measured in this systematic review. High glycemic loaded diets were a focus of the identified studies, whilst only one study specifically investigated carbohydrate consumption [248]. Only 3 studies utilised an *a posteriori* method of diet/nutritional measure. The utility of *a posteriori* approaches to investigating diet-disease associations has been well described [255]; however, a paucity of studies in this systematic review utilised statistical methods to define their dietary model.

Previous research has suggested that AD prevalence is related to diet [236]. Our systematic review has substantiated these findings with respect to the underlying pathophysiology of the hallmark AD biomarkers in plasma, CSF and the brain. Taken together with prior literature, these findings are consistent that modifiable lifestyle risk factors are associated with *in vivo* biomarker burden as well as manifestations of AD incidence.

This systematic review and meta-analysis provides support for the notion of a physiological mechanism of a diet/AD relationship that has been reported in several clinical studies. We identified substantial heterogeneity in study protocols, an array of dietary measures and multiple methods of tau/amyloid measurement. Our review was limited by a small number of identified studies. Studies were predominantly cross-sectional (14 out of 15), limiting causal inferences from the available published findings. Given several studies pooled their cohorts from the same larger populations, the possibility of participants overlapping in separate studies may bias the strength of our findings; however, all cohorts analysed in the systematic review were unique and were therefore not removed from qualitative analysis. Other mechanisms of neurodegeneration, such as white matter hyperintensities and atrophy may also contribute to the clinical manifestation of disease progression; however, were outside the scope of this review.

Future research would benefit from a focus on dietary patterns with robust study designs and larger samples to clarify the therapeutic utility of diet in AD. There was only one longitudinal investigation that investigated dietary patterns and AD biomarkers at two distinct time points and only 3 studies that utilised an *a posteriori* method of dietary analysis. Future longitudinal research is of particular relevance to the development of preventative measures, as well-defined cohorts allow researchers to eliminate mediators of a potential diet-AD mechanism. Our findings support a relationship between diet/nutrition and biomarkers of AD; however, given biomarkers can manifest decades

prior to symptomatology it remains to be seen how long-term dietary choices can impact subsequent pathology.

2.1.2.5 Conclusion

The findings from this systematic review and meta-analysis suggest adherence to a MeDi styled dietary pattern was associated with a reduction in AD biomarkers and subsequent pathology. Conversely, adherence to a high-glycemic, high saturated fat diet was associated with an increase in AD biomarker burden. These findings shed light on the pathophysiological processes that may underpin the association between diet and AD prevalence in previous studies.

2.2 Summary of current research

Results from diverse research methodologies indicate a substantial impact of diet on the underlying physiology and clinical manifestations of AD. Yusufov*, et al.* [141] found 50 out of 64 studies presented a significant relationship between diet and AD. However, only 6 studies identified by Yusufov*, et al.* [141] utilised biomarkers to suggest an AD diagnosis. Furthermore, there was a paucity of studies identified by Yusufov*, et al.* [141] that investigated dietary patterns and their associations to AD, whether clinical or neuropathological. Only 12 studies examined MeDi and three utilised factor analysis to extract dietary patterns *a posteriori*. Given the clinical manifestations of AD are preceded by decades of neuropathological change [256], there is clear impetus to examine the effect of dietary patterns on AD neuropathology.

3 METHODOLOGY & DESIGN

This chapter presents the methodology and design of the materials and protocols that have been utilised in the work described in this thesis. The following chapter will elaborate upon the summary of methods provided in results chapters, including the ethics, study population, clinical measures, dietary intake, neuropathological measurement, neuropsychological assessment, data analysis and ethics.

3.1 Introduction

The methodological integrity and transparency of research is of utmost importance. Throughout the production of this thesis, an appropriate level of ethics, governance and statistical analysis were endeavoured to be met. The following chapter elaborates upon the specific methodologies utilised in the production of this thesis: ethics, study population (cohort & recruitment, eligibility and exclusion), clinical measures (clinical, physical, genotypic), dietary measures (DQES v2, nutritional intake, dietary pattern score, DII score, MeDi score), neuropathological measures (Aβ), neuropsychological assessment (including the entire neuropsychological battery), characteristics of the cohort and data analysis (data management, confounding variables, statistical analysis).

3.2 Ethics

The Women's Healthy Ageing Project (WHAP) was initiated in 1990, initially named the Melbourne Women's Midlife Health Project (MWMHP). Ethics approval for the original study (MWMHWP) was granted by the University of Melbourne Human Research Ethics Committee (HREC) (Approval Number: 585008_A_O_89_0489). Over the following decades, ethics approval for the WHAP longitudinal study in subsequent years was granted by the University of Melbourne HREC (Approval Numbers: 931149X [92–99], 010528 and 010411 [02–09], 1034765 and 1339373 [since 2012]). The study was conducted in accordance with the National Health and Medical Council Ethical Conduct in Human Research and Declaration of Helsinki. Informed consent was obtained from all individuals prior to participant in the study.

Recognition of the ethics approval from the University of Melbourne HREC was provided to Australian Catholic University to enable production of the thesis. Human ethics application to access existing non-identifiable data was granted by Australian

Catholic University (Application Number: 2018-62) and renewed in 2019 (Application Number: 2018-62N). Data access was granted via BioGrid (Project Number: 201703/13).

3.2.1 Cohort

The WHAP, originally MWMHWP, was initiated to examine women's health from midlife into ageing. WHAP is an ongoing epidemiologically-sampled, prospective cohort study that has been collecting data on women's health for more than two decades. With almost 30 years of longitudinal prospective follow up in healthy ageing women, the study is unique in Australia. The study has been able to address critical questions regarding women's health and has published over 200 peer-reviewed publications, review articles, and books. A complete cohort profile has been published[257].

Drawing from an accumulated database of biological, physical, and psychosocial measures of women's experience throughout the menopausal transition and into ageing, WHAP has demonstrated a successful model of longitudinal prospective epidemiological research in women's health. WHAP has also established international collaborations and provided informative studies that have assisted in developing frameworks for national and international guidelines on women's health.

WHAP is in a unique position in Australia (Figure 21) given existing longitudinal epidemiological studies from midlife (Australian Longitudinal Study of Women's Health[258], Melbourne Collaborative Cohort Study[259], Household Income and Labour Dynamics in Australia[260]) lack longitudinal biomarker, neuropsychological, lifestyle and physical measures. Australian longitudinal cohort studies that collect neuropsychiatric measures and biomarkers (Memory and Ageing Study[261], Australian Longitudinal Study of Ageing[262], Australian Imaging, Biomarker and Lifestyle Study[263],

Path Through Life Study[264]) have only published information from participants aged over 60 or 70.

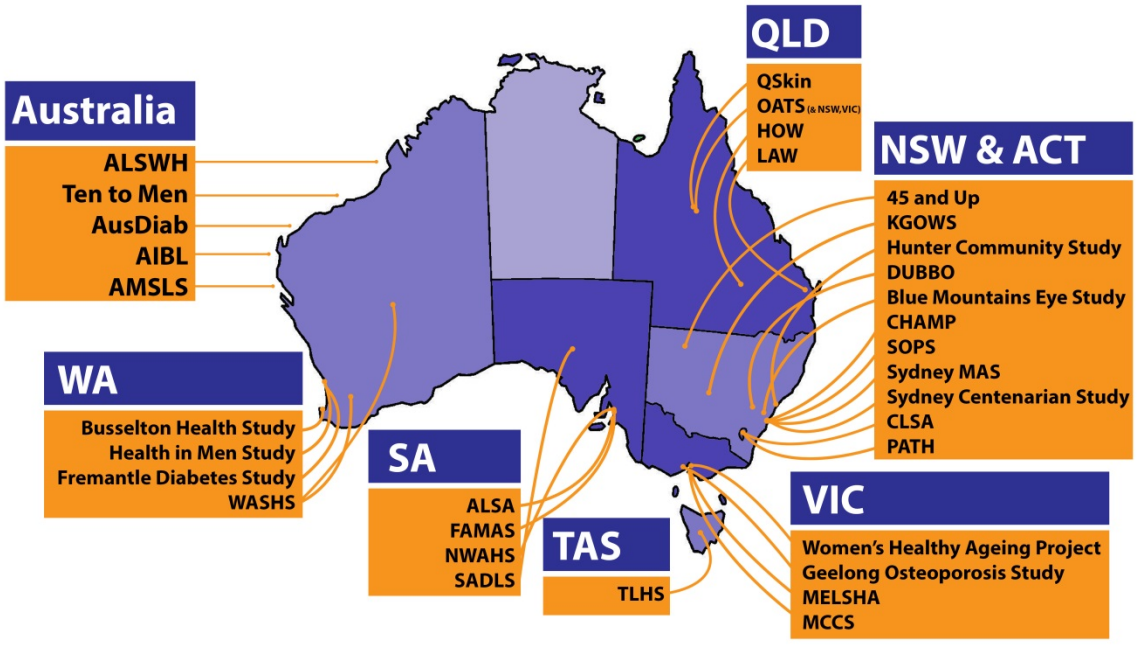

Figure 21: Summary of longitudinal cohort studies in Australia. Created by E.Hill

WHAP has been collecting longitudinal data at regular intervals since 1990 (Figure 22). WHAP has collected extensive validated measures of health and is unique in Australia in being able to incorporate biological, physical and neuropsychological measures to investigate the healthy ageing of women. The outcome variables utilised in WHAP cover six major themes: Quality of Life and Ageing, Mental and Cognitive Health, Cardiovascular Health, Musculoskeletal and Bone Health, Lifestyle, and Women's Health and Hormonal Transitions.

Throughout the WHAP's history, measurements have been conducted depending on their temporal relevance and clinical significance. Clinical interviews, lifestyle measures and psychological assessments have been conducted at all longitudinal follow up. Biomarker analysis, clinical examination and physical measures have been offered at every longitudinal follow up since 1992. Bone mineral density (BMD) Dual-Energy

X-ray Absorptiometry (DEXA) was offered in 1994, 1996 and 1998, whilst X-rays were conducted in 2002. Nutritional assessments were conducted in 1994, 1998, 2012 and 2014. For the purposes of this thesis, the 1998 and 2012 time points were utilised as these were the years where the DQES v2 was administered to WHAP participants.

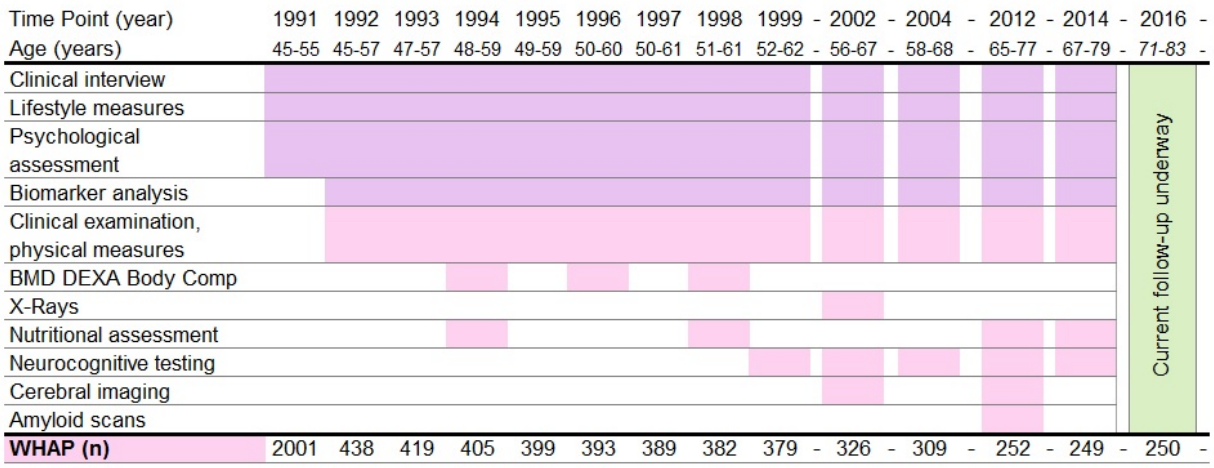

Figure 22: WHAP measures collected at each time point. From Szoeke, *et al.* [257]

3.2.2 Recruitment, eligibility and exclusion

In order to be eligible for the initial study (MWMHWP), participants had to be females aged between 45 - 55 years and born in Australia[265]. Participants were recruited by random digit dialling conducted by the Roy Morgan Centre (Figure 23). Using a computerised database of the Melbourne "White Pages" telephone directory, randomly selected numbers were called. The Roy Morgan Centre made 54,078 calls to Melbourne households, with 9,329 unanswered (17.3%). Of the 44,729 calls that were answered: 40,364 (90.2%) were terminated due to a household member not meeting inclusion criteria; 1,447 (3.2%) were terminated prior to establishing eligibility and 2,938 (6.6%) had a women eligible to be interviewed. Of the households with eligible women (2,938), 105 (3.6%) were unavailable due to being absent or ill and 2,833 (96.4%) were available. Of those available, 2,001 (70.6%) consented to take part in the baseline

telephone interview questionnaire and 832 (29.4%) did not consent. In 1992, eligible participants from the cross-sectional study were contacted and asked to participate in the annual longitudinal follow up. The longitudinal arm required an ability to determine menopausal status: had menses in the previous 3 months; had an intact uterus with at least one ovary and were not taking oral contraceptive or hormone therapy. A total of 438 (56.2%) or participants agreed to participate in follow up. Analysis of women who agreed to participate compared with those that did not found they: reported more than 12 years of education (34.3 and 24.3 % respectively, $p < 0.005$); more paid employment (71.4 and 63.0 % respectively, $p < 0.05$); had better comparative health (48.5 and 38.1 % respectively, $p < 0.005$); had exercised in the last week (68.0 and 58.1 % respectively, $p < 0.005$); had a Papanicolaou smear in the last year (58.3 and 50.0 % respectively, $p < 0.05$) and had undergone dilatation and curettage (46.5 and 38.4 % respectively, $p < 0.05$) [257]. Women in both groups were similar in age, BMI, marital status, parity, symptoms, well-being, stress, smoking status, alcohol consumption, surgical history, mammograms, tubal ligation, medication usage and suffering from premenstrual complaints[257].

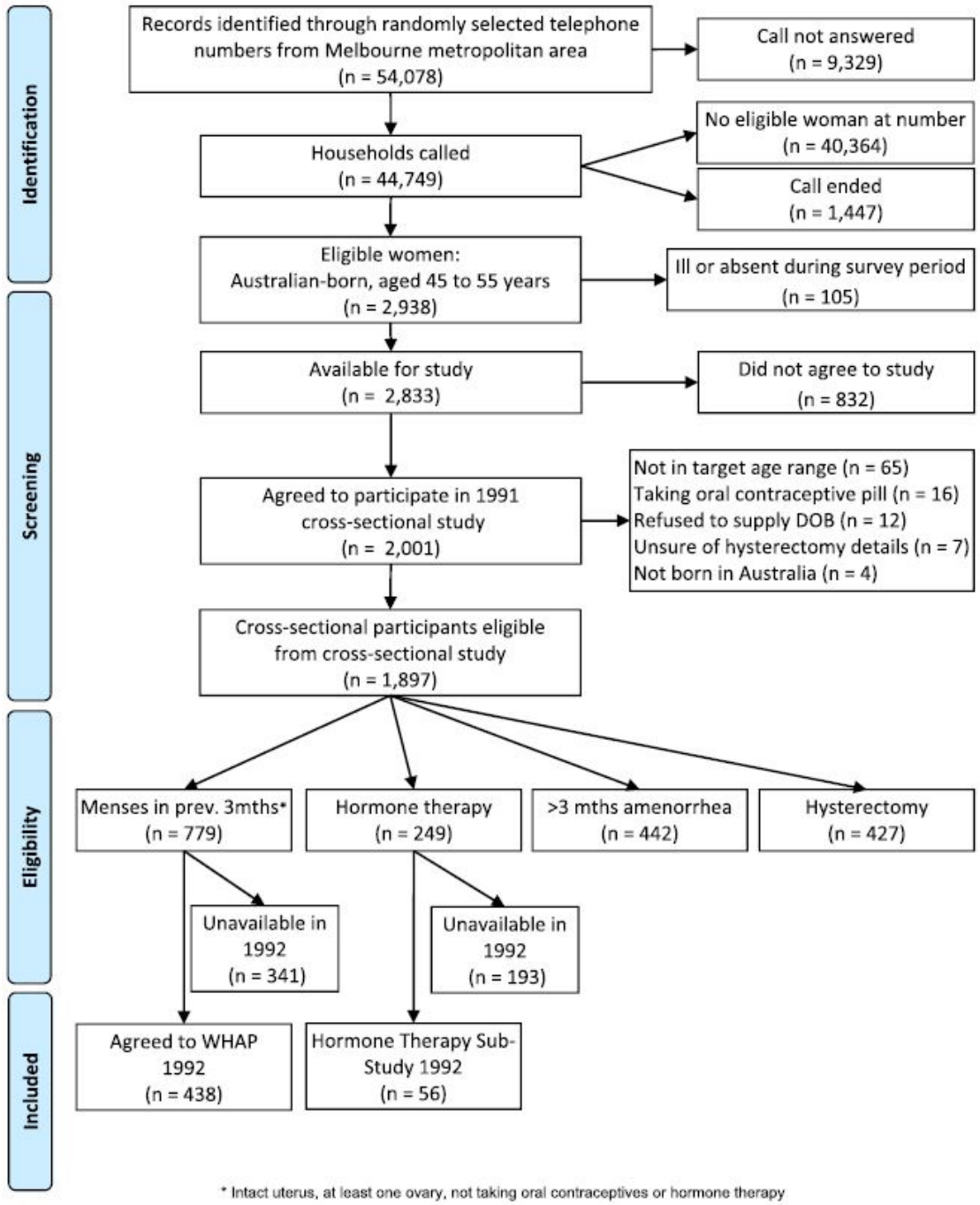

Figure 23: PRISMA chart outlining the selection of participants for the WHAP longitudinal cohort study. From Szoeke, *et al.* [257]

3.3 Clinical assessments

3.3.1 Study visits

All follow ups were completed in person. Participants were contacted by a researcher at each time point for confirm their continuation with WHAP and to schedule a follow up

assessment time. Upon confirmation of the study visit, participants were mailed the study information, participant consent form and self-reported questionnaires to be completed prior to study visit. Prior to their study visit, participants were asked to fast overnight for a minimum of 10 hours. At the study visit, written informed consent was obtained and blood samples were taken for biomarkers, genetic testing and biobank storage. Prior to the assessment commencing, participants were provided with breakfast. Assessment duration was approximately 4 hours[266]. Upon completion, participant data were transcribed and stored under unique de-identified serial numbers and paper records were kept securely.

3.3.2 Demographics

During the core questionnaire and the demographics questionnaire, participants were asked about sociological and demographic characteristics. These included items such as participant date of birth (format dd/mm/yyyy), age (in years), marital status (single, married, divorced, separated, widowed or other), education (in years, including primary school), employment status (yes/no: full time/part time), volunteer work (yes/no: full time/part time), languages spoken, number of pregnancies, number of births and number of children.

3.3.3 Physical measures

Standing height without shoes (to the nearest 0.1cm) and weight (to the nearest 0.1kg) were measured. Head, waist (at narrowest part of the trunk) and hip circumference were measured to the nearest cm. Participants' systolic and diastolic blood pressure was measured using a standardised blood pressure monitor. BMI calculated as weight in kilograms divided by height in meters squared.

Physical activity was recorded during assessments, reported as the number of days in the last 2 weeks participants engaged in any type of physical activity and in any type of

physical activity that resulted in shortness of breath. In 2012 the international Physical Activity Questionnaire was added to the protocol. The IPAQ measures the number of days (in last 2 weeks/month) a participant has engaged in physical activity for leisure or recreation[267]. Since 2012, several other physical performance scales have been added to the WHAP protocol; the Timed Up and Go [268], hand grip strength[269] and 4 meter walk[270].

3.3.4 Lifestyle

Participants were asked about their smoking status (number of cigarettes smoked per day, pack years calculated) and alcohol consumption (number of standard drinks in preceding 2 weeks) at each follow up. The Activities of Daily Living[271] was completed to assess changes in functional abilities, reported by the participant's partner, child or friend.

3.3.5 Blood measures

Fasting blood samples of 80mL were collected by peripheral venous puncture into multiple serum separator tubes. Within an hour of collection, the separator tubes were centrifuged, with 27mL forwarded to a clinical pathology laboratory for analysis. Biobank storage involved whole and fractioned blood samples stored in liquid nitrogen. Fractionation involved the blood being fractionated into the following components: serum, plasma, platelets, red blood cells, white blood cells. Fractionated components were stored in liquid nitrogen in 92 NUNC cryo-vial aliquots (0.25 to 1 mL). Blood samples were stored in lithium-heparin tubes, EDTA tubes (with prostaglandin E1) and serum tubes. Blood biomarker analyses included hormonal assays, lipid profiles, glucose sensitivity and genotyping (Table 7).

Hormonal Assays	Follicle stimulating hormone, estradiol, immunoreactive inhibin, luteinising hormone, testosterone, sex hormone binding globulin, free testosterone index, dehydroepiandrosterone, inhibin a, inhibin b, thyroid stimulating hormone, free thyroxine, free triiodothyronine
Lipid profile	Total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein
Chemistry and hematology	Glucose, insulin, c-reactive protein, homocysteine, vitamin b12, vitamin d, serum folate, red cell folate, sodium, potassium, chloride, bicarbonate, anion gap, creatinine, estimated glomerular filtration rate, urea, alanine transaminase, alkaline phosphatase, aspartate transaminase, gamma glutamyl transferase, bilirubin, albumin, total protein, iron, ferritin, transferrin, ceruloplasmin, calcium, magnesium, full blood count, erythrocyte count, haematocrit, haemoglobin, platelet count, red cell count, leucocyte count, white cell differential, red cell differential, packed cell volume, erythrocyte sediment rate
Genotyping	Estrogen receptor polymoprhism, androgen receptor polmorphism, cytochrome 17 polymorphism, and apolipoprotein-E COMT, and aromatase genotyping

Table 7: Blood biomarker analysis collected as part of WHAP protocol. From Szoeke, *et al.* [257]

3.3.6 Genotypic measures

Participants' APOE genotype was determined by direct sequencing (APOE ε2/ε4, APOE ε3/ε4, and APOE ε4/ε4). APOE genotype was also dichotomized as an APOE ε4 carrier or a non-carrier.

3.4 Dietary measures

3.4.1 Dietary Questionnaire for Epidemiological Studies Version 2

At both the 1998 and 2012 time points, WHAP participants completed the self-reported hardcopy version of the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2). The DQES v2 is a modified food frequency questionnaire that was developed by the Cancer Council of Victoria (CCV) in the 1980s to measure dietary intake of participants of the longitudinal Melbourne Collaborative Cohort Study. Participants self-report their usual dietary habits in the last 12 months. The DQES v2 covers 5 types of dietary intake; cereals/sweets/snacks, dairy/meat/fish, fruit, vegetables

and alcoholic beverages. The DQES v2 incorporates 80 food items (Table 8) with frequency response options on 74 of these items[272]. A sample DQES v2 is provided in Appendix 1.

3.4.2 Dietary intake calculation

Frequency of consumption relative to standard servings was estimated for 104 individual foods, mixed dishes and beverages including alcohol (Table 8). Estimated daily intake of foods was provided by the CCV.

Cereals, sweets and snacks	Wholemeal bread, rye bread, multigrain bread, high-fibre white bread, all-bran, bran flakes, Weet-Bix cereal, porridge, muesli, white bread, corn flakes, rice, pasta, crackers, sweet biscuits, cakes, chocolate, jam, sugar, nuts, peanut butter
Dairy, meat and fish	Beef, veal, lamb, pork, bacon, ham, sausage, salami, chicken, meat pies, pizza, hamburgers, fried fish, fish (non-fried), tinned fish, skim milk, reduced-fat milk, flavoured milk, low-fat cheese, ricotta cheese, full-cream milk, ice cream, yoghurt, hard cheese, firm cheese, soft cheese, cream cheese, soya milk, eggs, butter, butter-margarine blends, margarine, monounsaturated margarine, polyunsaturated margarine
Fruit	Oranges, apples, pears, bananas, melon, pineapple, strawberries, apricots, peaches, mango, avocado, tinned fruit, fruit juice
Vegetables	Chips (French fries), potatoes, carrot, pumpkin, capsicum, peas, green beans, baked beans, tofu, other beans, cabbage, cauliflower, broccoli, spinach, lettuce, cucumber, celery, beetroot, bean sprouts, onion, garlic, mushrooms, zucchini, tomatoes
Alcoholic beverages	Light beer, heavy beer, red wine, white wine, fortified wine, spirits

Table 8: Dietary intake groups from the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2)

3.4.3 Nutritional intake calculation

Completed DQES v2 questionnaires were posted to the CCV for analysis. Data collected using the DQES v2 in 1998 and 2012 was used to calculate nutrient intakes, based on the Australian nutrient composition data from NUTTAB95, which is based on the published Composition of Foods, Australia[273]. Analysis of the reported dietary intake was conducted by the Nutritional Assessment Office and converted the dietary intake into nutritional components (Table 9). Nutrients were estimated including total energy (kilojoules per day), total fat and fat subtypes, protein, carbohydrates and vitamins and minerals in grams, milligrams or micrograms per day.

Energy	Energy
Macronutrients	Carbohydrate, all Fat, Protein, Monounsaturated Fat, Polyunsaturated Fat, Saturated Fat, Cholesterol, Sugars, Starch, Fibre, individual fatty acids, Glycemic Index, Glycemic Load
Vitamins	Folate, Niacin, Niacin Equivalent, Retinol, Retinol Equivalent, Riboflavin, Thiamin, Vitamin C, Vitamin E, Carotenoids, Beta Carotene
Minerals	Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc
Alcohol	Alcohol (caloric intake from alcohol)

Table 9: Nutrients from foods calculated by the Nutritional Assessment Office of the Cancer Council Victoria

3.4.4 Dietary pattern score construction

Estimated daily intakes provided by the CCV were placed into 33 food groups defined *a priori*. Dietary patterns were extrapolated from food groupings using iterated principal factor analysis with oblique varimax rotation due to the presumed inter-collinearity and non-independence of dietary patterns. Dietary patterns were extrapolated for both time points utilising both absolute and energy adjusted estimated daily intakes. Energy adjusted means were calculated by dividing the individual food item by the estimated daily energy intake. Pattern factor loading matrices were analysed to provide appropriate subjective nomenclature.

3.4.5 Dietary Inflammatory Index calculation

Dietary Inflammatory Index (DII) scores of WHAP participants were calculated at both 1998 and 2012 time points. Scores were calculated by The Cancer Prevention and Control Program, University of South Carolina.

Constructing the DII score involved an extensive literature review to identify publications on specific foods and nutrients and their associations with six a priori defined inflammatory biomarkers: Interleukin (IL)-1β, IL-4, IL-6, IL-10, TNF-α and C-

reactive protein (CRP). Publications that met the criteria were indexed and scored to derive component-specific inflammatory effect scores for the 27 DII items (Table 10). Details on the development[274], validation[275] and utility[276] have been described previously.

DII items for WHAP	Alcohol, beta carotene, carbohydrate, cholesterol, energy, fat, fiber, garlic, iron, magnesium, monounsaturated fatty acids, niacin, omega 3, omega 6, onion, protein, polyunsaturated fatty acids, riboflavin, saturated fat, thiamin, vitamin C, vitamin C, zinc
Other DII items	Vitamin B12, vitamin B16, caffeine, eugenol, folic acid, ginger, saffron, selenium, transunaturated fatty acids, turmeric, vitamin A, vitamin D, tea, flavanols, flavonones, anthocyanidins, isoflavones, pepper, thyme, oregano, rosemary

Table 10: Items included and excluded in the calculation of the Dietary Inflammatory Index for WHAP participants

3.5 Neuropathological measures

3.5.1 Beta-amyloid

In the 2012 follow-up, all WHAP participants were offered the opportunity to have cerebral imaging. Aβ deposition was measured via *in vivo* F-18 Florbetaben positron emission tomography (PET), a radioligand that enables the quantification of cerebral Aβ[277]. PET scans were conducted at the Austin Health Centre for PET in Victoria, Australia. Participants received 250 MBq of 18F-florbetaben intravenously, with a 20-minute acquisition commencing 90 minutes after injection. Standardized uptake values (SUVs) were calculated for all brain regions examined, and standard uptake value ratios

(SUVRs) were generated by normalizing regional SUVs by the cerebellar cortex with atrophy correction from structural magnetic resonance imaging. Neocortical SUVR, a global index of Aβ burden, is expressed as the average SUVR of the area weighted mean. Area-weighted means were calculated for each participant by averaging the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. For WHAP participants in 2012, PET SUVR displayed a positive skew that was rectified using 1/square transformation.

3.6 Neuropsychological assessment

3.6.1 Consortium to Establish a Registry for Alzheimer's Disease

Since 1998, WHAP participants completed the neuropsychological test of cognition entitled the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[278]. CERAD is a 10 item supraspan word list score sensitive to early changes associated with Alzheimer's dementia[279,280]. As part of the in-person core questionnaire, WHAP participants were read 10 items and then asked to repeat back as many of the items from the list as they could remember. For trials 2 and 3 the order of words spoken was altered (reversed or halved), and the delayed recall was conducted after 3 minutes. Testing protocol is provided in Appendix 5.

CERAD protocol was followed at both 1998 and 2012 WHAP time points and was utilised to calculate the CERAD Savings score. CERAD Savings score has been described as a valid indicator of cognitive ability[281] and has been suggested to be the most reliable index to differentiate cognitively normal individuals from AD[282]. CERAD Savings scores were calculated by dividing the delayed recall by the score on the third learning trial.

3.7 Characteristics of the cohort

3.7.1 Demographics

In 1998, a total of 436 (retention: 89%[257]) participants underwent WHAP assessments. By 2012, a total of 252 (retention: 58%[257]) participants were seen. Due to various circumstances throughout the years, participants have not completed every scale at every time point. The following is a summary of the demographics of the WHAP participants in 1998 and 2012, preserving as many data points as possible.

		1998	N
Age			
	Mean age in years (± SD)	55.7 ± 0.1	436
Marital Status			
	Single	16 (3.7%)	436
	Married	340 (78.0%)	
	Divorced	39 (8.9%)	
	Separated	12 (2.8%)	
	Widowed	21 (4.8%)	
	Other	8 (1.8%)	
BMI			
	Underweight (<18.5kg/m^2)	0 (0.0%)	345
	Healthy weight (>= 18.5 and < 25kg/m^2)	139 (40.3%)	
	Overweight (>=25 and <30kg/m^2)	130 (37.7%)	
	Obese (>=30kg/m^2)	76 (22.0%)	
Smoking status			
	Current non-smoker	378 (86.7%)	436
	Current smoker	58 (13.3%)	
Paid Employment			
	Currently in paid employment	158 (36.2%)	436
	Currently not in paid employment	278 (63.8%)	
Volunteer Work			
	Currently doing unpaid work	239 (54.8%)	436
	Currently not doing unpaid work	197 (45.2%)	
Cognition			
	Mean CERAD Savings score (± SD)	92.7% ± 15.6	334

Table 11: Sociodemographic statistics for all WHAP participants who attended the 1998 time point and data is available (n = 436)

		2012	N
Age			
	Mean age in years (± SD)	70.2 ± 0.2	252
Marital Status			
	Single	6 (2.5%)	241
	Married	154 (63.9%)	
	Divorced	30 (12.5%)	
	Separated	6 (2.5%)	
	Widowed	34 (14.1%)	
	Other	11 (4.6%)	
Education			
	Mean education in years (± SD)	12.2 ± 0.2	245
BMI			
	Underweight (<18.5kg/m^2)	0 (0%)	238
	Healthy weight (>= 18.5 and < 25kg/m^2)	73 (30.7%)	
	Overweight (>=25 and <30kg/m^2)	95 (39.9%)	
	Obese (>=30kg/m^2)	70 (29.4%)	
Smoking status			
	Current non-smoker	223 (92.9%)	240
	Current smoker	17 (7.1%)	
Paid Employment			
	Currently in paid employment	46 (19.3%)	238
	Currently not in paid employment	192 (80.7%)	
Volunteer Work			
	Currently doing unpaid work	137 (57.6%)	238
	Currently not doing unpaid work	101 (42.4%)	
Grandchildren			
	Currently has grandchildren	203 (85.3%)	238
	Currently does not have grandchildren	35 (14.7%)	
Cognition			
	Mean CERAD Savings score (± SD)	63.7% ± 28.7	234
APOE Genotype			
	ε2/ ε3	27 (10.71%)	217

ε2/ ε4	8 (3.2%)
ε3/ ε3	123 (48.8%)
ε3/ ε4	55 (21.8%)
ε4/ ε4	4 (1.6%)

Table 12: Sociodemographic statistics for all WHAP participants who attended the 2012 time point and data is available (n = 252)

3.7.2 Classification of participants

WHAP was established as a healthy control cohort; however, throughout the course of the study participants may have progressed into stages along the progression of Alzheimer's disease. Throughout the course of the study, participants who display signs of mild cognitive impairment through clinical and neuropsychological assessment are referred to neuropsychological panel. The following is a summary of the classification, both clinical and neuropathological, of the WHAP participants in 1998 and 2012, preserving as many data points as possible.

3.7.2.1 Clinical

At the inaugural visit, all WHAP participants were classified as healthy controls, with no sign of cognitive decline consistent with a diagnosis of AD or early onset AD. Throughout the near 30 years the study has been running, participants that display evidence of mild cognitive impairment in clinical and neuropsychological assessment have their complete files sent to review by a clinical panel. The clinical review panel review all available medical, psychiatric, and neuropsychological information to confirm the cognitive health (HC, MCI, AD) of individuals, and the panel was blinded to amyloid imaging results. In 2012 [257], classifications were comprehensively conducted on all WHAP participants that attended study visits (n = 252) and the results are displayed in Table 13.

	N	%
Healthy control	218	85.7
MCI	29	12.2
AD	5	2.1

Table 13: Cognitive classification of all WHAP participants in 2012

3.7.2.2 Neuropathological

Amyloid neuropathology was measured via PET scan in 2012. Out of 252 participants in 2012, 124 (49.0%) underwent ^{18}F amyloid PET imaging. PET SUVR distribution displayed a positive skew visually represented in Figure 24. Summary statistics stratified by presence of the APOE-ε4 allele are displayed in Table 14 and visually depicted in Figure 25.

	N	^{18}F PET SUVR	CI	Min	Max
All participants	124	1.1409	1.1030 – 1.1788	0.940	2.06
APOE Genotype					
APOE-ε4 positive	41	1.2532	1.0614 – 1.1158	0.950	2.06
APOE-ε4 negative	80	1.0886	1.1604 – 1.3460	0.940	1.60

Table 14: Summary statistics for mean PET SUVR for all WHAP participants (n = 124), stratified by presence of APOE-ε4 allele

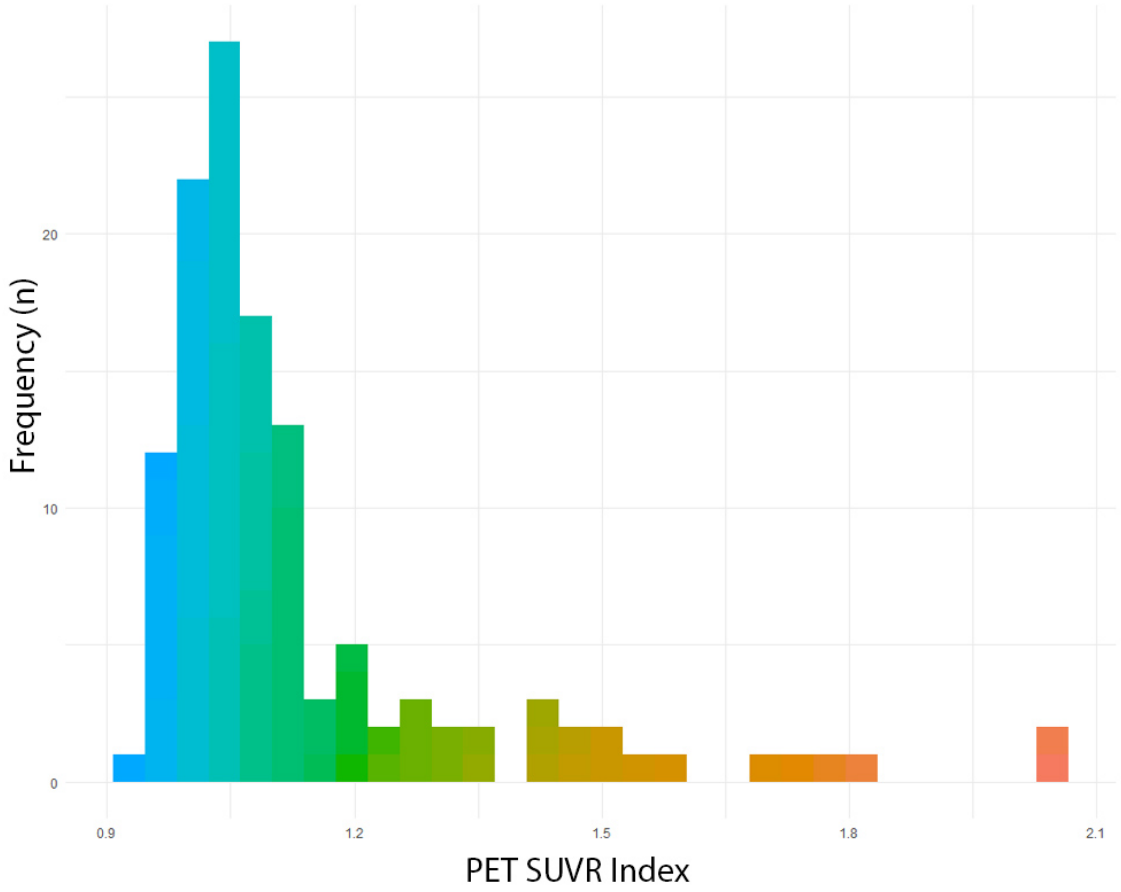

Figure 24: Frequency histogram of raw PET SUVR values for all available WHAP participants (n = 124)

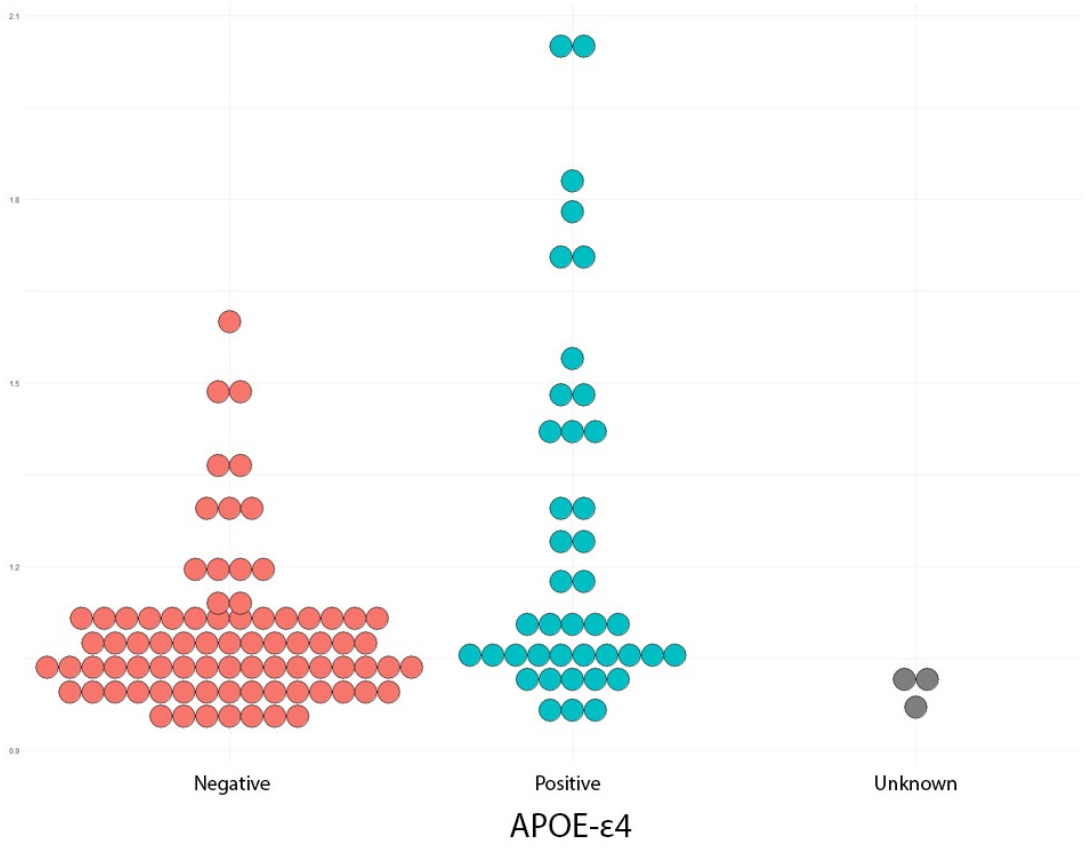

Figure 25: Frequency dotplot of raw PET SUVR values for all available WHAP participants stratified by presence of APOE-ε4 allele

3.7.3 Dietary Intake

3.7.3.1 Food items

WHAP participants estimated mean daily intake of food items calculated by the CCV are displayed in Table 15. Data presented include all WHAP participants that completed the DQES v2 at each time point: 349 in 1998 and 235 in 2012.

Food item	1998 Mean	1998 CI	2012 Mean	2012 CI
All Bran (g/day)	2.2	1.5 - 2.9	2.1	1.4 - 2.8
Bran flakes (g/day)	2.8	1.9 - 3.7	1.7	1.0 - 2.4
High fibre white bread (g/day)	7.1	4.8 - 9.4	5.8	3.2 - 8.5
Muesli (g/day)	9.9	7.9 – 12.0	12.6	9.7 - 15.6
Multigrain bread (g/day)	35.2	30.5 - 39.8	21.7	17.8 - 25.7

Porridge (g/day)	26.0	19.9 - 32.1	33.6	26.6 - 40.6
Rye bread (g/day)	7.5	4.7 - 10.4	6.8	4.1 - 9.5
Weet Bix (g/day)	5.0	3.8 - 6.2	3.4	2.5 - 4.3
Wholemeal bread (g/day)	15.8	12.1 - 19.5	17.1	12.9 - 21.3
Corn flakes (g/day)	2.9	2.1 - 3.7	2.7	1.7 - 3.6
Crackers (g/day)	7.0	6.0 - 7.9	7.1	6.0 - 8.3
Pasta (g/day)	34.4	29 - 39.8	16.5	14.6 - 18.3
Rice (g/day)	26.1	22.1 – 30.0	15.8	12.0 - 19.5
White bread (g/day)	11.1	7.9 - 14.3	7.8	4.9 - 10.8
Beef (g/day)	23.4	21.0 - 25.9	18.6	16.3 - 20.8
Lamb (g/day)	11.3	10.0 - 12.7	10.0	8.7 - 11.4
Pork (g/day)	3.0	2.5 - 3.5	3.6	2.8 - 4.3
Veal (g/day)	2.5	1.8 - 3.1	2.0	1.2 - 2.9
Bacon (g/day)	1.9	1.6 - 2.2	2.5	2.1 - 2.9
Salami (g/day)	1.6	1.2 – 2.0	1.4	1.1 - 1.7
Sausages (g/day)	5.5	3.2 - 7.7	3.7	2.8 - 4.6
Chicken (g/day)	20.6	17.3 - 23.9	14.6	13.2 – 16.0
Hamburger (g/day)	4.0	3.3 - 4.8	4.4	2.4 - 6.3
Meat pies (g/day)	11.9	10.3 - 13.5	11.7	9.6 - 13.7
Pizza (g/day)	7.7	6.6 - 8.8	7.2	5.8 - 8.5
Fried fish (g/day)	3.3	2.6 - 4.1	3.3	2.2 - 4.3
Fish (g/day)	12.8	11.1 - 14.6	13.9	11.2 - 16.5
Tinned fish (g/day)	9.3	7.4 - 11.3	8.3	6.9 - 9.6
Chips (g/day)	8.2	6.9 - 9.5	8.8	7.4 - 10.3
Potatoes (g/day)	28.7	26.3 - 31.2	23.4	20.5 - 26.3
Capsicum (g/day)	2.7	2.4 - 3	2.6	2.2 – 3.0
Carrots (g/day)	10.2	9.3 - 11.1	8.6	7.9 - 9.4
Pumpkin (g/day)	10.3	9.2 - 11.3	10.1	8.7 - 11.5
Baked beans (g/day)	4.1	3.3 - 4.9	3.5	2.7 - 4.2
Green beans (g/day)	7.4	6.7 - 8.1	8.6	7.6 - 9.5
Other beans (g/day)	2.5	1.8 - 3.2	2.1	1.6 - 2.6
Peas (g/day)	6.7	6.0 - 7.4	6.0	5.1 - 6.8
Tofu (g/day)	2.2	1.6 - 2.9	1.3	0.9 - 1.8
Broccoli (g/day)	10.0	9.1 - 10.9	10.8	9.7 - 12
Cabbage (g/day)	5.1	4.5 - 5.6	4.5	3.7 - 5.3

Cauliflower (g/day)	7.6	6.8 - 8.4	8.3	7.2 - 9.4
Lettuce (g/day)	7.6	7.0 - 8.2	8.6	7.9 - 9.4
Spinach (g/day)	3.9	3.3 - 4.6	4.2	3.6 - 4.8
Beansprouts (g/day)	0.7	0.6 - 0.8	0.3	0.2 - 0.4
Beetroot (g/day)	2.7	2.2 - 3.1	2.6	2.2 – 3.0
Celery (g/day)	3.8	3.5 - 4.2	4.0	3.5 - 4.5
Cucumber (g/day)	4.2	3.8 - 4.7	5.0	4.4 - 5.6
Garlic (g/day)	0.4	0.4 - 0.4	0.5	0.4 - 0.6
Mushrooms (g/day)	2.9	2.6 - 3.3	2.1	1.8 - 2.5
Onion (g/day)	4.5	4.1 - 4.9	4.0	3.5 - 4.4
Zucchini (g/day)	5.3	4.5 – 6.0	5.0	4.3 - 5.7
Tomatoes (g/day)	11.2	10.1 - 12.3	13.0	11.5 - 14.6
Apples (g/day)	40.2	36.2 - 44.2	26.1	22.9 - 29.3
Apricots (g/day)	8.2	6.6 - 9.9	4.7	3.5 - 5.9
Avocado (g/day)	5.3	4.4 - 6.3	7.1	6.0 - 8.1
Bananas (g/day)	45.2	41.1 - 49.4	38.6	34 - 43.3
Mango (g/day)	4.6	3.6 - 5.7	4.7	3.5 - 5.8
Melon (g/day)	17.5	14.9 - 20	14.2	11.7 - 16.6
Oranges (g/day)	54.7	48.3 - 61.2	46.1	38.9 - 53.3
Peaches (g/day)	11.0	9.0 - 12.9	9.5	7.5 - 11.6
Pears (g/day)	20.8	17.9 - 23.7	21.3	17.7 - 24.8
Pineapple (g/day)	4.9	3.9 - 5.8	3.8	3.0 - 4.7
Strawberries (g/day)	7.1	6.1 - 8.2	8.7	7.2 - 10.2
Tinned fruit (g/day)	12.9	9.2 - 16.6	9.8	7.3 - 12.2
Cakes (g/day)	10.9	9.0 - 12.7	10.0	8.5 - 11.6
Sweet biscuits (g/day)	5.8	4.8 - 6.7	6.9	5.7 - 8.1
Flavoured milk drink (g/day)	0.8	0.5 - 1.1	0.9	0.6 - 1.2
Low fat cheese (g/day)	3.7	2.8 - 4.7	2.3	1.3 - 3.3
Reduced fat milk (g/day)	146.5	125.8 - 167.1	149.8	124.5 - 175.1
Ricotta or cottage cheese (g/day)	1.1	0.6 - 1.6	0.8	0.3 - 1.3
Skim milk (g/day)	71.5	56.1 - 86.9	73.6	54.6 - 92.6
Cream cheese (g/day)	0.1	0.0 - 0.2	0.2	0.0 - 0.3
Firm cheese (g/day)	6.0	5.0 - 7.1	7.5	6.0 – 9.0
Full cream milk (g/day)	45.4	32.9 - 57.9	59.4	42.5 - 76.3

Hard cheese (g/day)	0.5	0.3 - 0.7	0.6	0.3 - 0.8
Ice cream (g/day)	6.0	4.9 - 7.1	7.4	5.4 - 9.4
Soft cheese (g/day)	0.9	0.5 - 1.3	2.1	1.4 - 2.8
Yoghurt (g/day)	50.8	44.3 - 57.3	65.1	56.7 - 73.4
Soya milk (g/day)	47.3	33.4 - 61.2	32.0	19.8 - 44.3
Chocolate (g/day)	4.4	3.5 - 5.3	6.1	4.9 - 7.3
Jam (g/day)	3.8	3.2 - 4.3	3.3	2.7 - 3.9
Sugar (g/day)	7.7	6.4 – 9.0	5.4	4.2 - 6.5
Crisps (g/day)	1.8	1.4 - 2.1	1.4	1 - 1.8
Nuts (g/day)	2.5	2.0 – 3.0	3.9	3.2 - 4.7
Peanut butter (g/day)	0.9	0.6 - 1.1	0.8	0.6 - 1.1
Eggs (g/day)	12.0	11.1 – 13.0	16.5	15.0 – 18.0
Fruit juice (g/day)	54.5	46.5 - 62.4	24.7	19.3 – 30.0
Butter and margarine blends (g/day)	2.2	1.5 - 2.9	2.0	1.3 - 2.6
Margarine (g/day)	1.5	1.0 - 2.1	1.0	0.5 - 1.4
Monounsaturated margarine (g/day)	2.6	1.8 - 3.4	1.1	0.6 - 1.6
Polyunsaturated margarine (g/day)	6.1	5.1 - 7.1	4.9	3.8 - 5.9
Heavy beer (g/day)	6.0	1.0 – 11.0	13.5	2.0 - 29.1
Light beer (g/day)	15.3	4.3 - 26.4	4.9	0.8 – 9.0
Red wine (g/day)	40.4	29.8 – 51.0	67.6	50.4 - 84.7
White wine (g/day)	67.4	54.3 - 80.5	77.2	60.6 - 93.7
Fortified wines (g/day)	6.7	4.2 - 9.2	4.0	1.6 - 6.3
Spirits (g/day)	5.2	3.5 - 6.9	2.7	1.5 - 3.9

Table 15: Estimated mean daily intakes (95% CI) of DQES v2 food items for all available WHAP participants in 1998 (n = 349) and 2012 (n = 235)

3.7.3.2 Food groups

Food items were grouped into 33 food groups defined a priori and similar to those used by others [283,284]. WHAP participants estimated mean daily intake of food groups are

presented in Table 16. Data presented include all WHAP participants that completed the DQES v2 at each time point: 349 in 1998 and 235 in 2012.

	1998 Mean	**1998 CI**	**2012 Mean**	**2012 CI**
Whole grains (g/day)	111.5	103.2 - 119.8	104.9	96.2 - 113.7
Refined grains (g/day)	81.4	72.8 – 90.0	49.9	43.9 - 55.8
Red meats (g/day)	40.3	36.7 - 43.8	34.2	30.8 - 37.7
Processed meats (g/day)	9.0	6.5 - 11.4	7.7	6.4 - 8.9
Poultry (g/day)	20.6	17.3 - 23.9	14.6	13.2 – 16.0
Takeaway foods (g/day)	23.6	21.3 - 25.9	23.2	19.5 – 27.0
Fried fish (g/day)	3.3	2.6 - 4.1	3.3	2.2 - 4.3
Other fish (g/day)	22.1	19.2 - 25.1	22.2	19.0 - 25.3
Fried potatoes (g/day)	8.2	6.9 - 9.5	8.8	7.4 - 10.3
Other potato (g/day)	28.7	26.3 - 31.2	23.4	20.5 - 26.3
Yellow or red vegetables (g/day)	23.1	21.5 - 24.8	21.3	19.6 - 23.1
Legumes (g/day)	23.0	21.3 - 24.7	21.4	19.7 - 23.2
Cruciferous vegetables (g/day)	22.6	21.0 - 24.3	23.6	21.4 - 25.8
Leafy green vegetables (g/day)	11.5	10.6 - 12.4	12.8	11.9 - 13.8
Other vegetables (g/day)	24.5	23.0 – 26.0	23.5	21.8 - 25.1
Tomato (g/day)	11.2	10.1 - 12.3	13.0	11.5 - 14.6
Fresh fruit (g/day)	219.5	206.8 - 232.2	184.7	171.1 - 198.3
Canned fruit (g/day)	12.9	9.2 - 16.6	9.8	7.3 - 12.2
Cakes, biscuits, sweet pastries (g/day)	16.6	14.2 – 19.0	16.9	14.7 - 19.1
Low-fat dairy products (g/day)	223.6	202.7 - 244.6	227.4	202.0 - 252.8
Full- fat dairy products (g/day)	109.8	96.5 - 123.1	142.3	123.8 - 160.8
Soya milk (g/day)	47.3	33.4 - 61.2	32.0	19.8 - 44.3

Confectionery (g/day)	4.4	3.5 - 5.3	6.1	4.9 - 7.3
Added sugar (g/day)	11.5	10.1 - 12.9	8.6	7.3 – 10.0
Crisps (g/day)	1.8	1.4 - 2.1	1.4	1.0 - 1.8
Nuts (g/day)	3.3	2.7 – 4.0	4.7	3.9 - 5.6
Eggs (g/day)	12.0	11.1 – 13.0	16.5	15.0 – 18.0
Fruit juice (g/day)	54.5	46.5 - 62.4	24.7	19.3 – 30.0
Saturated spreads (g/day)	3.7	2.8 - 4.5	2.9	2.1 - 3.7
Unsaturated spreads (g/day)	8.7	7.5 - 9.9	6.0	4.9 - 7.1
Alcohol - beer (g/day)	21.3	9.2 - 33.5	18.4	2.4 - 34.5
Alcohol - wine (g/day)	107.8	90.8 - 124.8	144.8	121.4 - 168.1
Alcohol - spirits (g/day)	11.9	8.9 - 14.9	6.7	4.0 - 9.4

Table 16: Estimated mean daily intakes (95% CI) of food groups for all available WHAP participants in 1998 (n = 349) and 2012 (n = 235)

3.7.3.3 Nutrients

Nutrient intakes for WHAP participants were estimated by the CCV based upon completed DQES v2 questionnaires. Estimated mean daily energy intake and nutrient intakes are displayed in . Data presented include all WHAP participants that completed the DQES v2 at each time point: 349 in 1998 and 235 in 2012.

	1998 Mean	**1998 CI**	**2012 Mean**	**2012 CI**
Energy (kJ/day)	5797.1	5575.8 - 6018.3	5311.6	5101.4 - 5521.9
All Fat (g/day)	53.2	50.7 - 55.8	51.5	49.0 – 54.0
Saturated Fat (g/day)	20.2	19.1 - 21.3	20.9	19.7 - 22.1
Monounsaturated Fat (g/day)	18.6	17.6 - 19.5	17.9	17.0 - 18.8
Polyunsaturated Fat (g/day)	9.6	9.1 - 10.1	8.3	7.8 - 8.8
Protein (g/day)	67.4	64.7 - 70.1	63.0	60.3 - 65.6
Carbohydrate (g/day)	160.3	154.2 - 166.3	139.5	133.8 - 145.2

Sugars (g/day)	75.9	72.9 – 79.	69.5	66.3 - 72.7
Starch (g/day)	83.3	79.5 - 87.1	69.0	65.6 - 72.4
Fibre (g/day)	18.9	18.1 - 19.7	16.6	15.9 - 17.4
Beta Carotene (ug/day)	2076.6	1958.4 - 2194.7	1867.0	1768.1 - 1965.8
Calcium (mg/day)	833.6	801.0 - 866.2	850.3	811.3 - 889.3
Cholesterol (mg/day)	189.8	180.3 - 199.4	204.2	193.5 - 214.9
Folate (ug/day)	233.2	223.6 - 242.8	215.2	206.1 - 224.4
Iron (mg/day)	10.0	9.5 - 10.4	9.2	8.7 - 9.6
Magnesium (mg/day)	242.4	233.2 - 251.5	228.6	219.2 - 238.1
Niacin (mg/day)	15.7	14.9 - 16.5	13.9	13.2 - 14.5
Niacin Equivalent (mg/day)	28.8	27.6 - 30.1	26.2	25.0 - 27.3
Phosphorus (mg/day)	1230.8	1184.5 - 1277	1200.4	1151.3 - 1249.5
Potassium (mg/day)	2376.8	2298.1 - 2455.6	2237.6	2159.7 - 2315.5
Retinol (ug/day)	277.8	263.1 - 292.5	277.0	260.6 - 293.4
Retinol Equivalent (ug/day)	624.8	598.0 - 651.5	588.9	564.0 - 613.8
Riboflavin (mg/day)	2.0	1.9 - 2.1	1.9	1.9 – 2.0
Sodium (mg/day)	1830.0	1749.2 - 1910.8	1631.3	1561.4 - 1701.2
Thiamin (mg/day)	1.3	1.2 - 1.3	1.1	1.1 - 1.2
Vitamin C (mg/day)	110.9	104.8 - 116.9	89.9	84.9 - 94.9
Vitamin E (mg/day)	5.3	5.0 - 5.5	5.0	4.8 - 5.2
Zinc (mg/day)	8.7	8.3 – 9.0	8.1	7.7 - 8.4

Table 17: Estimated mean daily intakes (95% CI) of nutrients for all available WHAP participants in 1998 (n = 349) and 2012 (n = 235)

3.7.4 Attrition

Throughout this ongoing longitudinal study, attrition of WHAP participants is inevitable. Participants have left the study due to various reasons, such as moving out of the Melbourne metropolitan area, missing assessments or due to an inability to contact

the participant. A summary of reasons for attrition at each WHAP time point is provided in Table 18.

Year	WHAP (n)	Moved	Illness	Too busy	Family reasons	Too stressful	Simply refused	Unable to contact	Missed review	Resumed participation at review
1992	438	-2	0	-2	-1	0	-5	-3	-6	0
1993	419	-2	0	-3	0	0	-2	-1	-11	0
1994	405	-2	0	-1	0	0	-4	0	-7	0
1995	399	0	0	0	0	0	0	0	-10	4
1996	393	-1	0	-1	0	0	-3	-2	0	1
1997	389	-2	0	0	0	0	0	-1	-2	1
1998	382	0	0	-1	0	0	-1	-1	-4	0
1999	379	0	-2	0	0	0	-1	-1	-1	2
2002	326	0	-2	0	-2	-1	-1	-5	-64	0
2004	309	-2	-4	-1	0	-2	-1	-2	-29	16
2012	252	0	0	0	0	0	0	0	-82	26

Table 18: Reason for attrition of WHAP participants at each time point and the number of participants lost to follow up

Death is an inevitable consequence of ageing and a number of WHAP participants have passed away during the course of the study. For the 1,897 participants eligible for cross-sectional study (Figure 23), 1,804 were not deceased in 2017 (Table 19).

	Not deceased (n=1,804)	**Cancer (n=49)**	**Cardiovascular (n=12)**	**Respiratory (n=7)**	**Infection (n=2)**	**Dementia (n=4)**	**Other[a] (n=11)**	**Unknown cause (n=8)**
Principal cause of death								
Brain cancer	-	3 (6.1%)	-	-	-	-	-	-
Breast cancer		13 (26.5%)						
Lung cancer		7 (14.3%)						
Ovarian cancer		3 (6.1%)						
Skin cancer		5 (10.2%)						
Leukaemia/lymphoma		3 (6.1%)						
Other organ cancer		12 (24.5%)						
Cancer, NOS		3 (6.1%)						
Myocardial infarction	-	-	1 (12.5%)	-	-	-	-	-
Heart disease			1 (12.5%)					
Mitral valve prolapse			1 (12.5%)					
Intracranial haemorrhage			1 (12.5%)					
Subdural haemorrhage			1 (12.5%)					
Hypercholesterolaemia			1 (12.5%)					
Stroke, non-haemorrhage			2 (25.0%)					
Asthma	-	-	-	2 (28.6%)	-	-	-	-
COPD, acute exacerbation				1 (14.3%)				
COPD w/ LRTI				2 (28.6%)				
COPD, unspecified				2 (28.6%)				
Enterocolitis	-	-	-	-	1 (50.0%)	-	-	-
Necrotising fasciitis					1 (50.0%)			
Dementia, Alzheimer's			-	-	-	1 (25.0%)	-	-
Dementia, frontotemporal						1 (25.0%)		
Dementia, unspecified						2 (50.0%)		

Epileptic spasms	-	-	-	-	-		1 (9.1%)	-
Parkinson's disease							1 (9.1%)	
Diabetes							2 (18.2%)	
Fall/accident							2 (18.2%)	
Other[a]							5 (45.5%)	
Year of death;	-	2003.7	2007.1	2007.9	2011.5	2013.8	2006.9	2016.1
mean (SD; range)		(7.3; 1991 – 2015)	(7.3; 1996 – 2015)	(8.4; 1992 – 2015)	(2.1; 2010 – 2013)	(1.0; 2013 – 2015)	(5.6; 1995 – 2014)	(0.4; 2016 – 2017)
Age at baseline, years;	49.7	50.4	50.6	50.6	48.0	51.3	48.9	49.9
mean (SD; range)	(3.1; 45 – 55)	(3.3; 45 – 55)	(3.1; 45 – 55)	(2.2; 48 – 54)	(0; 48 – 48)	(2.2; 49 – 54)	(3.7; 45 – 55)	(2.2; 47 – 53)
Age at death, years;	-	63.8	67.3	68.0	69.4	74.8	65.2	75.8
mean (SD; range)		(8.3; 47.2 – 79.5)	(7.9; 57.6 – 77.7)	(9.2; 51.3 – 77.2)	(1.8; 68.1 – 70.6)	(3.2; 71.4 – 79.0)	(6.9; 54.1 – 74.2)	(2.1; 73.5 – 79.1)
Case category								
WHAP core	419 (23.2%)	7 (14.3%)	3 (25.0%)	0	2 (100.0%)	2 (50.0%)	3 (27.3%)	2 (25.0%)
HRT substudy	51 (2.8%)	1 (2.0%)	1 (8.3%)	0	0	0	1 (9.1%)	0
Recontact	52 (2.9%)	1 (2.0%)	0	0	0	0	0	1 (12.5%)
Baseline only	1,282 (71.1%)	40 (81.6%)	8 (66.7%)	7 (100.0%)	0	2 (50.0%)	7 (63.6%)	5 (62.5%)
BMI								
Under/normal	1,167 (66.6%)	36 (73.5%)	6 (54.6%)	5 (71.4%)	2 (100.0%)	4 (100.0%)	7 (70.0%)	4 (50.0%)
Overweight	427 (24.4%)	9 (18.4%)	4 (36.4%)	1 (14.3%)	0	0	2 (20.0%)	1 (12.5%)
Obese	158 (9.0%)	4 (8.2%)	1 (9.1%)	1 (14.3%)	0	0	1 (10.0%)	3 (37.5%)
Baseline menopausal status								
Pre-meno	448 (24.8%)	8 (16.3%)	4 (33.3%)	2 (28.6%)	2 (100.0%)	0	5 (45.5%)	2 (25.0%)
Perimeno	292 (16.2%)	11 (22.5%)	1 (8.3%)	0	0	2 (50.0%)	0	1 (12.5%)
Late perimeno	83 (4.6%)	1 (2.0%)	0	0	0	1 (25.0%)	0	2 (25.0%)
Post-meno	339 (18.8%)	9 (18.4%)	2 (16.7%)	2 (28.6%)	0	0	3 (27.3%)	0
HRT	237 (13.1%)	7 (14.3%)	2 (16.7%)	1 (14.3%)	0	0	2 (18.2%)	0

Hysterectomy	405 (22.5%)	13 (26.5%)	3 (25.0%)	2 (28.6%)	0	1 (25.0%)	1 (9.1%)	3 (37.5%)
Duration pre/post-menopause at baseline, y								
-10	8 (1.8%)	0	0		0	0	0	0
-5	176 (39.3%)	3 (37.5%)	3 (75.0%)		0	1 (50.0%)	1 (33.3%)	1 (50.0%)
-3	120 (26.8%)	1 (12.5%)	1 (25.0%)		0	0	1 (33.3%)	1 (50.0%)
-2	64 (14.3%)	2 (25.0%)	0		1 (50.0%)	1 (50.0%)	0	0
-1	47 (10.5%)	1 (12.5%)	0		1 (50.0%)	0	0	0
0	33 (7.4%)	1 (12.5%)	0		0	0	1 (33.3%)	0
Ever smoked at baseline								
No	1,081 (74.9%)	22 (66.7%)	6 (85.7%)	1 (33.3%)	0	3 (100.0%)	4 (66.7%)	2 (66.7%)
Yes	363 (25.1%)	11 (33.3%)	1 (14.3%)	2 (66.7%)	1 (100.0%)	0	2 (33.3%)	1 (33.3%)
Current smoker at baseline								
No	1,444 (80.0%)	33 (67.4%)	7 (58.3%)	3 (42.9%)	1 (50.0%)	3 (75.0%)	6 (54.6%)	3 (37.5%)
Yes	360 (20.0%)	16 (32.7%)	5 (41.7%)	4 (57.1%)	1 (50.0%)	1 (25.0%)	5 (45.5%)	5 (62.5%)
Marital status at baseline								
Married/living w/ partner	1,420 (78.7%)	42 (85.7%)	8 (66.7%)	4 (57.1%)	2 (100.0%)	2 (50.0%)	9 (81.8%)	6 (75.0%)
Separated	247 (13.7%)	5 (10.2%)	2 (16.7%)	1 (14.3%	0	2 (50.0%)	1 (9.1%)	1 (12.5%)
Divorced	64 (3.6%)	1 (2.0%)	0	2 (28.6%)[†]	0	0	1 (9.1%)	1 (12.5%)
Widowed	73 (4.1%)	1 (2.0%)	2 (16.7%)[†]	0	0	0	0	0
Employed at baseline								
No	642 (35.6%)	23 (46.9%)	4 (33.3%)	5 (71.4%)	1 (50.0%)	1 (25.0%)	5 (45.5%)	3 (37.5%)
Yes	1,162 (64.4%)	26 (53.1%)	8 (66.7%)	2 (28.6%)	1 (50.0%)	3 (75.0%)	6 (54.6%)	5 (62.5%)

Education completed at baseline (in years)								
≥8	935 (51.8%)	23 (46.9%)	9 (75.0%)	7 (100.0%)	1 (50.0%)	2 (50.0%)	6 (54.6%)	3 (37.5%)
9 – 12	428 (23.7%)	15 (30.6%)	3 (25.0%)	0	1 (50.0%)[†]	1 (25.0%)	3 (27.3%)	3 (37.5%)
>12	441 (24.5%)	11 (22.5%)	0	0	0	1 (25.0%)	2 (18.2%)	2 (25.0%)
					0			

a Other causes of death: Diverticulitis of intestine with perforation & abscess; obesity (unspecified); spondylolysis, multiple; syndrome of inappropriate secretion of antidiuretic hormone; systemic lupus erythematosus with organ/system involvement.

b. Data presented is current as of the latest linkage project with Births Deaths and Marriages (July 2017)

Table 19: Demographics and causes of death for the WHAP participants

3.8 Data Analysis

3.8.1 Data management

De-identified electronic data was stored on secured, encrypted networks and was accessed via Biogrid. All participant data was stored under unique identifier serial numbers and paper records of test results are kept securely in a locked compactus located in the Centre for Medical Research at the Royal Melbourne Hospital, Victoria, Australia.

3.8.2 Confounding variables

Analysis dependent confounding variables were defined *a priori*. Depending on previous research, independent & dependent variables and the hypotheses being investigated, confounding variables were included in descriptive statistics and statistical models at all stages of analysis where appropriate. Age (in years), education (in years), BMI, energy intake (kJ/day), APOE-ε4 (binary or specific [ε2/ε2, ε2/ε3, ε3/ε3, ε3/ε4, ε2/ε4 or ε4/ ε4]), cognition (CERAD Savings), smoking status, marital status, employment status and volunteer status were common covariates utilised in analyses.

3.8.3 Statistical analysis

All statistical analyses were conducted in IBM SPSS v22 (SPSS Inc., Chicago, IL, USA), STATA v14 (StataCorp LLC, College Station, TX, USA) or R v3.4 (R Foundation for Statistical Computing, Vienna, Austria) on Windows 7 Enterprise operating system (Microsoft Corporation, Albuquerque, NM, USA).

Based upon respective research methodologies and hypotheses to be investigated, all SPSS, STATA and R syntax was overseen and approved by a qualified biostatistician. Unless otherwise stated, all *p* values were adjusted for multiple analyses using the

Bonferroni method. Unless otherwise stated, a p value of 0.05 or smaller determined a significant result for all analyses. Where variables displayed a skew that violated assumptions for statistical analyses, adjustments were made utilising an appropriate model fit. Generalised linear models were tested and goodness of fit was determined using Akaike's and Bayesian Information Criterion.

4 LONGITUDINAL NUTRITIONAL CHANGES

This chapter presents an investigation of prospective changes in nutritional changes in participants of the WHAP. Differences in nutritional intakes, DII, MeDi adherence, sociodemographic and physical measures were analysed in 1998 and 2012. This chapter presents significant changes in the intake of energy and several nutrients over 14 years of follow up. Between 1998 and 2012, changing nutritional indices for WHAP participants were consistently in the direction of a poorer diet.

This chapter contains a modified version of the following publication [285]:

Hill E, Hodge A, Clifton P, Shivappa N, Hebert JR, Dennerstein L, Campbell S, Szoeke C. Longitudinal nutritional changes in aging Australian women. Asia Pacific Journal of Clinical Nutrition. 2019;28:139.

4.1 Introduction

With global populations surviving longer, research is shifting focus toward the promotion of healthy aging. A growing body of research acknowledges the importance of diet for the maintenance of health during aging [286]. Because there is a natural decrease in caloric intake with aging [287,288], the investigation of nutritional sufficiency in aging adults is particularly important in order to maximize quality of life and the independence of individuals in the community. Over the last few decades there has been a great change in the cooking and eating habits of the Australian population. Greater consumption of energy-dense foods and larger portion sizes have resulted in excessive energy intake, leading to overweight and obesity [289]. A comparative investigation into the 1995 and 2011-2012 National Nutrition Surveys found *per capita* decreases in fruit and vegetable intake but increases in *per capita* wine, cocoa, nuts and seafood intake [290]. Furthermore, the substantial differences in health and longevity between men and women may be attributed to nutritional status [291-294]. According to the most recent Australian Health Survey (2011-2012), the proportion of overweight and obese adult women has risen from 54.7% in 2007-2008 to 56.2% in 2011-2012 [295]. As dietary intakes, along with BMI, are among the leading risk factors contributing to the burden of disease in Australasia [296,297], research into the changing dietary patterns of aging Australians is important.

The sociodemographic circumstances of aging individuals can impact nutritional inadequacies that may contribute to loss of function and disease. Widowhood is common in older women and has been associated with a decrease in dietary quality [298,299]. Widowhood also places older adults at a greater risk for depression [300], which has been associated with several chronic diseases such as diabetes [301], cancer [302], and

coronary heart disease [303]. Dietary variety also is decreased for those living alone [304]. Education is consistently associated with higher diet quality in elderly men and women [305] and a systematic review found dietary intake changed as a function of employment transition to retirement [306].

In contrast with conventional nutritional approaches that focus on a single nutrient, dietary pattern analysis examines the diet overall [307]. Dietary patterns reflect interrelated actions of multiple food components and have been shown to represent dietary intake and to be consistently associated with all-cause, cardiovascular and cancer mortality [308]. A growing body of evidence [142] supports the beneficial role of the Mediterranean Diet (MD), characterized by high consumption of fruits, vegetables, nuts, legumes, cereals and fish; a moderate intake of alcohol; and a low intake of meat and dairy.

The dietary inflammatory index (DII®) has been established as an indicator of an individual's dietary inflammatory potential. Based on an extensive literature review of the pro- or anti-inflammatory properties of foods and nutrients, the DII identified 45 items with reported associations with biomarkers of inflammation and derives a total score based on the individual intakes of these food parameters [274]. A higher DII score reflects a more pro-inflammatory diet while a lower DII score is more anti-inflammatory. The method of deriving the DII as an overall marker of dietary quality based on a physiological mechanism, inflammation, is in contrast to previously used methods which have assessed adherence to a pre-defined dietary pattern (e.g., Med Diet Score, Alternate Healthy Eating Index) or have used statistical techniques to find patterns within dietary data (e.g., principal components analysis/factor analysis or cluster analysis [255]). Diets having a low DII score are characterized by high intakes of fruit and vegetables and other plant-based foods, and would be considered 'healthy' according to other methods [309,310]. Higher DII scores have been associated with

inflammatory biomarkers and HOMA insulin resistance [275,276,311]. Prospective studies have reported positive associations between DII scores and myocardial infarction [312], lung cancer [309], prostate cancer [313], breast cancer [314] and metabolic syndrome [315]. DII scores also have been shown to be associated with all-cause, cardiovascular and cancer mortality [316].

Despite the expanding use of dietary patterns in nutritional epidemiology [317-319], relatively few studies have investigated temporal changes in dietary patterns. Adherence to a MD pattern remained stable in women in the Nurse's Health Study over 14 years [320]. Mishra*, et al.* [321] used a modified factor analysis to derive dietary patterns from three time points for men and women in the UK. For women, three patterns were identified, over time there was an increase in consumption of foods from ethnic foods and alcohol; and fruit, vegetables and dairy patterns; while consumption of foods from the meat, potatoes and sweet food pattern decreased [321]. In a prospective investigation over 4 years, Mulder*, et al.* [322] found age and socioeconomic status influenced lifestyle behaviors, including alcohol consumption and diet. Cross-sectional research in a prospective cohort study in Melbourne found greater MD adherence was associated with a reduction in mortality among Anglo-Celts and Greek-Australians[323]. Knowledge of the longitudinal stability and influencers of dietary intake could aid researchers in suggesting dietary interventions, tracking trends in dietary stability and minimizing the necessity for frequent data collection. Furthermore, investigating the temporal nature of dietary patterns and disease course could elucidate the ideal window for the timing of interventions.

To the best of our knowledge, only one study has investigated longitudinal DII changes in women [324]. In the observational study of the Women's Health Initiative, DII scores fell from -1.14(+/-2.58) at baseline to -1.50(+/-2.60) three years later, indicating a move

to a slightly less inflammatory diet. Decreases in DII scores over time were associated with demographic and lifestyle characteristics, including having a normal BMI, being highly educated and of Asian/Pacific Island or European-American origin rather than African-American or Hispanic origin. Despite the large numbers (around 76,000 women) this study does not provide information on dietary change over the longer term. Our aim in this paper is to describe prospective changes in dietary patterns and nutrient intakes in a sample of mid to late-life women in the Melbourne metropolitan area**.** We hypothesized temporal changes in indices reflecting dietary choices would be in the direction of a poorer diet.

4.2 Materials and methods

4.2.1 Cohort

This study utilised data collected as part of the 1998 and 2012 follow-up (Table 20) of the ongoing cohort study; The Women's Healthy Aging Project (WHAP). WHAP is a longitudinal, epidemiological study of 438 mid-life women in the Melbourne (Australia) metropolitan area. Participants were originally recruited in 1991 by random digit dialing and were eligible for the study if they were Australian-born, aged 45-55 years, had menstruated in the three months prior to recruitment and were not on any form of hormone replacement therapy [266]. The WHAP has been approved by the University of Melbourne Human Research Ethics Committee (HREC: 931149X , 1034765 & 1750632.1). The study was conducted in accordance with the National Health and Medical Council Ethical Conduct in Human Research and Declaration of Helsinki. A full WHAP study protocol has been previously published [266].

		1998	2012
Age			
	Mean age in years	55.1	69.8
Marital Status			
	Single	6 (3.5%)	5 (2.9%)
	Married	132 (76.3%)	107 (61.8%)
	Divorced	18 (10.4%)	24 (13.9%)
	Separated	5 (2.9%)	5 (2.9%)
	Widowed	8 (4.6%)	25 (14.5%)
	Other	4 (2.3%)	7 (4%)
Education		Highest Education in 1998	
	Primary school	3 (1.7%)	
	Secondary school	88 (50.9%)	
	Technical or commercial/TAFE	8 (4.6%)	
	Tertiary Diploma	29 (16.8%)	
	University or CAE degree	45 (26.0%)	
BMI			
	Underweight (18.5kg/m^2)	0 (0%)	0 (0%)
	Healthy weight (>= 18.5 and < 25kg/m^2)	64 (37%)	52 (30.1%)
	Overweight (>=25 and <30kg/m^2)	68 (39.3%)	69 (39.9%)
	Obese (>=30kg/m^2)	41 (23.7%)	52 (30.1%)
Smoking status			
	Current smoker	23 (13.3%)	14 (8.1%)
	Current non-smoker	150 (86.7%)	159 (91.9%)
Paid Employment			
	Currently in paid employment	122 (70.5%)	40 (23.1%)
	Currently not in paid employment	51 (29.5%)	133 (76.9%)
Volunteer Work			
	Currently doing unpaid work	96 (55.5%)	95 (54.9%)
	Currently not doing unpaid work	77 (44.5%)	78 (45.1%)

Expressed are numbers (%). Percentage is of time point population total

Table 20: Sociodemographic characteristics of Women's Healthy Ageing Project with data in 1998 and 2012

4.2.2 Materials and procedure

4.2.2.1 Nutritional data

Participants completed a validated FFQ (DQES v2) prior to their assessments in 1998 and again in 2012. The Dietary Questionnaire for Epidemiological Studies Version 2 (DQES) was developed by the Cancer Council of Victoria (CCV) and incorporates 80 food items with frequency response options on 74 of these items [325]. The DQES v2 covers five types of dietary intake; cereals/sweets/snacks, dairy/meat/fish, fruit, vegetables and alcoholic beverages. Data collected using the DQES v2 was used to calculate nutrient intakes, based on the Australian nutrient composition data from NUTTAB95, which is based on the published Composition of Foods, Australia [273]. The data from the questionnaires were used to compute food group intakes MD and DII scores. Daily food intakes for all 104 foods were estimated (in grams/day) from the DQES v2 and grouped into 33 food groups defined *a priori* (Table 21) in a similar method to those used by others [326,327].

Food Group	Items in the DQES v2
Whole grains	All bran, bran flakes, high fibre white bread, muesli, multigrain bread, porridge, rye bread, Weet-Bix, wholemeal bread
Refined grains	Corn flakes, crackers, pasta, rice, white bread
Red meats	Beef, lamb, pork, veal
Processed meats	Bacon, salami, sausages
Poultry	Chicken
Takeaway foods	Hamburger, meat pies, pizza
Fried fish	Fried fish
Other fish	Fish (non-fried), tinned fish
Fried potatoes	Chips (French fries)
Other potato	Potatoes
Yellow or red vegetables	Capsicum, carrots, pumpkin
Legumes	Baked beans, green beans, other beans, peas, tofu
Cruciferous vegetables	Broccoli, cabbage, cauliflower
Leafy green vegetables	Lettuce, spinach
Other vegetables	Bean sprouts, beetroot, celery, cucumber, garlic, mushrooms, onion, zucchini
Tomato	Tomatoes
Fresh fruit	Apples, apricots, avocado, bananas, mango, melon, oranges, peaches, pears, pineapple, strawberries
Canned fruit	Tinned fruit
Cakes, biscuits, sweet pastries	Cakes, sweet biscuits
Low-fat dairy products	Flavoured milk drink, low-fat cheese, reduced fat milk, ricotta cheese, cottage cheese, skim milk
Full- fat dairy products	Cream cheese, firm cheese, full-cream milk, hard cheese, ice cream, soft cheese, yoghurt
Soya milk	Soya milk
Confectionery	Chocolate
Added sugar	Jam, sugar
Crisps	Crisps
Nuts	Nuts, peanut butter
Eggs	Eggs
Fruit juice	Fruit juice
Saturated spreads	Butter, butter-margarine blends, margarine
Unsaturated spreads	Monounsaturated margarine, polyunsaturated margarine

Alcohol - beer	Heavy beer, light beer
Alcohol - wine	Red wine, white wine
Alcohol - spirits	Fortified wines, spirits
Food Group	Items in the DQES v2

Table 21: Food groupings from Dietary Questionnaire for Epidemiological Studies Version 2

4.2.2.2 Mediterranean diet adherence

Adherence to a Mediterranean Diet (MD) was assessed using a scoring tool devised by Sofi*, et al.* [142], based on the original method by Trichopoulou*, et al.* [160]. The MD score is based on intake of nine dietary components: vegetables, legumes, fruit dairy, cereals, meat and meat products, fish, alcohol and olive oil. This scoring system uses three-tiers, with zero, one or two points allocated for each component. The mean value of the weighted medians from all the cohort studies analyzed by Sofi*, et al.* [142] ±2 standard deviations were used to define the three tiers with zero points for the lowest intakes of fruit, vegetables, legumes, cereals and fish, the highest intakes of meat and meat products, and dairy products, and highest or lowest alcohol intakes. While fruit, vegetables, legumes, cereals and fish scored 2 points for the highest intake, meat and dairy products scored 2 points for the lowest intakes, and alcohol scored 2 points for the mid-level intake. The component regarding olive oil consumption was modified to a proxy measure, monounsaturated fatty acids to saturated fatty acids (MUFA:SFA) ratio for our study, due to the FFQ not including questions on oil intake [272]. Population-specific tertiles of MUFA:SFA were created (cut-points of 0.83 and 1.00 for 1998 and 0.79 and 0.95 for 2012) and 2 points were assigned to the highest MUFA:SFA ratio. This literature-based MD score allocates an individual score of 0 points for minimal adherence or 18 for maximal adherence.

4.2.2.3 Dietary inflammatory index

Details on the development [274] and validation [275,276] of the DII have been described previously. Briefly, an extensive literature review identified publications on specific foods and nutrients and their associations with six inflammatory biomarkers; IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP. These publications were indexed and scored to derive component-specific inflammatory effect scores. The 27 components used to calculate DII scores in this study are presented in Table 22.

Nutrients	1998	1998 CI	2012	2012 CI	P-value
Energy (kJ/day) †	5772.56	5438.25-6106.86	5322.81	5072.14-5573.48	0.004**
All Fat (% of kJ/day) †	33.39%	32.53-34.24	35.47%	34.72-36.23	<0.001***
Saturated Fat (% of kJ/day)	12.7%	12.21-13.19	14.18%	13.71-14.65	<0.001***
Monounsaturated Fat (% of kJ/day) †	11.61%	11.24-11.98	12.39%	12.07-12.72	0.001**
Polyunsaturated Fat (% of kJ/day) †	6.01%	5.63-6.39	5.78%	5.43-6.12	0.344
Fiber (g/mJ) †	3.31	3.18-3.44	3.20	3.20 (3.09-3.32)	0.122
Carbohydrate (% of kJ/day) †	47.39%	46.50-48.28	44.76%	43.95-45.56	<0.001***
Protein (% of kJ/day) †	19.97%	19.53-20.41	20.46%	20.00-20.91	0.047
Cholesterol (mg/mJ) †	32.64	31.30-33.98	38.74	37.05-40.44	<0.001***
Beta Carotene (ug/kJ) †	0.36	0.33-0.38	0.38	0.35-0.41	0.100
Folate (ug/mJ) †	40.80	39.19-42.42	42.09	40.49-43.68	0.152
Thiamin (mg/mJ) †	0.22	0.21-0.23	0.22	0.21-0.22	0.483
Niacin (mg/mJ) †	2.71	2.62-2.8	2.65	2.57-2.73	0.208
Niacin Equivalent (mg/mJ) †	4.99	4.87-5.11	5.00	4.90-5.11	0.859
Riboflavin (mg/mJ) †	0.35	0.33-0.36	0.37	0.36-0.39	0.003**
Vitamin C (mg/mJ) †	19.40	18.12-20.68	17.77	16.63-18.91	0.013
Vitamin E (mg/mJ) †	0.92	0.88-0.95	0.97	0.93-1.01	0.028
Retinol (ug/kJ) †	46.57	43.95-49.18	50.68	48.15-53.21	0.006*
Retinol Equivalent (ug/mJ) †	106.13	101.85-110.41	114.42	109.24-119.59	0.003**
Iron (mg/mJ) †	1.74	1.68-1.80	1.77	1.7-1.83	0.480
Zinc (mg/mJ) †	1.51	1.48-1.55	1.54	1.51-1.58	0.099
Magnesium (mg/mJ) †	42.32	41.04-43.60	43.91	42.75-45.07	0.018

Sodium (mg/mJ)	318.79	312.36-325.22	309.34	302.61-316.08	0.015
α-linolenic acid n-3(g/mJ) †	0.14	0.13-0.14	0.13	0.12-0.13	0.067
Long chain n-3 (g/mJ) †	0.05	0.044-0.060	0.057	0.051-0.064	0.782
Omega n-6 (g/mJ) †	1.34	1.25-1.43	1.32	1.24-1.41	0.798
Alcohol (grams) (% of kJ/day) †	8.32%	6.85-9.8	9.42%	7.44-11.41	0.168
Heavy beer‡ (g/mJ)	1.41	-0.75-3.58	1.35	-0.79-3.49	0.573
Light beer‡ (g/mJ)	2.38	0.58-4.18	1.10	0.06-2.13	0.036
Red wine‡ (g/mJ)	10.10	7.36-12.83	15.84	10.64-21.05	0.017
Spirits‡ (g/mJ)	0.90	0.54-1.26	0.55	0.33-0.76	0.048
White wine‡ (g/mJ)	15.14	11.01-19.26	15.70	10.54-20.86	0.798
Garlic‡ (g/day) †	0.09	0.08-0.11	0.10	0.09-0.12	0.229
Onion‡ (g/day)†	0.82	0.73-0.92	0.81	0.71-0.91	0.764
DII	-0.60	-0.80- -0.41	-0.46	-0.67-0.25	0.150
MD Adherence Score	7.14	6.78-7.51	5.95	5.65-6.26	<0.001***

*p< 0.01, **p<0.005, ***p<0.001. †Item included in the DII calculation. ‡ mL of beverage. Daily intakes expressed. Macronutrients expressed as % of total energy kJ/day. P values presented are unadjusted.

Table 22: Energy density means, 95% CI and t-test for difference for intakes of WHAP

4.2.2.4 Covariates

Participants' education in years was collected in 1998. Marital, employment and smoking status were collected during assessments in both 1998 and 2012 and used as categorical variables. Weight and height were measured in 1998 and 2012 and BMI calculated as weight in kilograms divided by height in meters squared. Underweight was defined as $<18.5kg/m^2$, healthy weight as $\geq 18.5kg/m^2$ and $<25kg/m^2$, overweight as $\geq 25kg/m^2$ and $<30kg/m^2$, obese as $\geq 30kg/m^2$.

4.2.2.5 Data analysis

IBM SPSS Statistics 22® software was used to conduct the statistical analyses for the present research. Multiple analyses were adjusted using the Bonferonni method. Nutrient intakes were adjusted for energy by dividing individual nutrient intake (e.g. g/day) by total energy intake (kJ/day). Macronutrients were expressed as percentages of total energy intake and were calculated according to energy contents of fat 37kJ/g, protein 17kJ/g, carbohydrate 16kJ/g and alcohol 29kJ/g. Categorical characteristics were described using cross-tabulation and frequency tables and compared between groups using the Pearson's Chi-squared or Fisher's exact test as appropriate. Paired sample t-tests were conducted to evaluate individual differences in energy, absolute intakes, energy-adjusted intakes, DII and MD between 1998 and 2012. Estimated energy-adjusted food group intakes in 1998 and 2012 were compared using paired sample t-tests (Table 23). A conceptual diagram illustrating the background, study design, and key findings of this study is presented in Figure 26.

Food Group	1998 Mean	1998 CI	2012 Mean	2012 CI	P-value
Whole grains (g/day)	108.55	97.51 - 119.58	101.3	91.05 - 111.55	0.2114
Refined grains (g/day)	87.09	72.98 - 101.2	51.6	43.87 - 59.33	<0.001***
Red meats (g/day)	39.97	35.67 - 44.27	34.41	30.53 - 38.29	0.0118*
Processed meats (g/day)	10.42	5.7 - 15.14	7.39	5.91 - 8.86	0.1207
Poultry (g/day)	19.86	15.98 - 23.74	15.15	13.51 - 16.79	0.0137*
Takeaway foods (g/day)	25.18	21.74 - 28.63	24.28	19.38 - 29.17	0.7485
Fried fish (g/day)	3.31	2.2 - 4.41	3.02	2.22 - 3.81	0.6543
Other fish (g/day)	23.5	18.6 - 28.39	22.83	18.95 - 26.71	0.7899
Fried potatoes (g/day)	7.39	6.14 - 8.64	8.76	7.13 - 10.39	0.1018
Other potato (g/day)	27.41	24.05 - 30.77	24.58	20.94 - 28.21	0.1768
Yellow or red vegetables (g/day)	21.9	19.89 - 23.91	22.4	20.3 - 24.51	0.6973
Legumes (g/day)	20.95	18.73 - 23.18	21.62	19.55 - 23.69	0.5859
Cruciferous vegetables (g/day)	21.95	19.65 - 24.25	23.09	20.66 - 25.52	0.3948
Leafy green vegetables (g/day)	11.67	10.23 - 13.11	13.14	11.97 - 14.32	0.0874
Other vegetables (g/day)	24.47	22.51 - 26.43	24.01	22.23 - 25.79	0.6623
Tomato (g/day)	11.7	10.09 - 13.3	12.99	11.19 - 14.8	0.2512
Fresh fruit (g/day)	217.05	199.92 - 234.17	187.35	171.27 - 203.44	<0.001***
Canned fruit (g/day)	10.18	6.53 - 13.82	10.34	7.34 - 13.33	0.9441
Cakes, biscuits, sweet pastries (g/day)	19.34	15.58 - 23.1	17.88	15.21 - 20.54	0.4616
Low-fat dairy products (g/day)	218.31	190.38 - 246.23	219.67	190.61 - 248.73	0.9267
Full-fat dairy products (g/day)	105.25	87.97 - 122.53	135.68	115.65 - 155.7	0.0063*

Soya milk (g/day)	47.11	27.34 - 66.88	36.27	21.23 - 51.31	0.3122
Confectionery (g/day)	4.63	3.22 - 6.04	6.36	4.85 - 7.87	0.0656
Added sugar (g/day)	11.77	9.67 - 13.88	8.12	6.59 - 9.65	<0.001***
Crisps (g/day)	1.88	1.38 - 2.38	1.52	1.02 - 2.02	0.2942
Nuts (g/day)	2.82	2.04 - 3.6	4.89	3.85 - 5.93	<0.001***
Eggs (g/day)	11.49	10.15 - 12.82	16.55	14.82 - 18.29	<0.001***
Fruit juice (g/day)	49.91	39.48 - 60.33	24.26	18.07 - 30.44	<0.001***
Saturated spreads (g/day)	3.68	2.49 - 4.87	2.37	1.58 - 3.15	0.0573
Unsaturated spreads (g/day)	8.07	6.46 - 9.69	6.05	4.77 - 7.33	0.0200*
Alcohol beverage - beer (g/day)	21.51	6.38 - 36.65	11.68	1.4 - 21.95	0.0564
Alcohol beverage - wine (g/day)	126.27	104.42 - 148.13	148.81	120.4 - 177.22	0.0694
Alcohol beverage - spirits (g/day)	13.42	9.44 - 17.4	7.18	4.09 - 10.27	<0.001***

Table 23: Mean daily intakes and 95% CIs for DQES v2 food groupings of WHAP participants by year of dietary survey

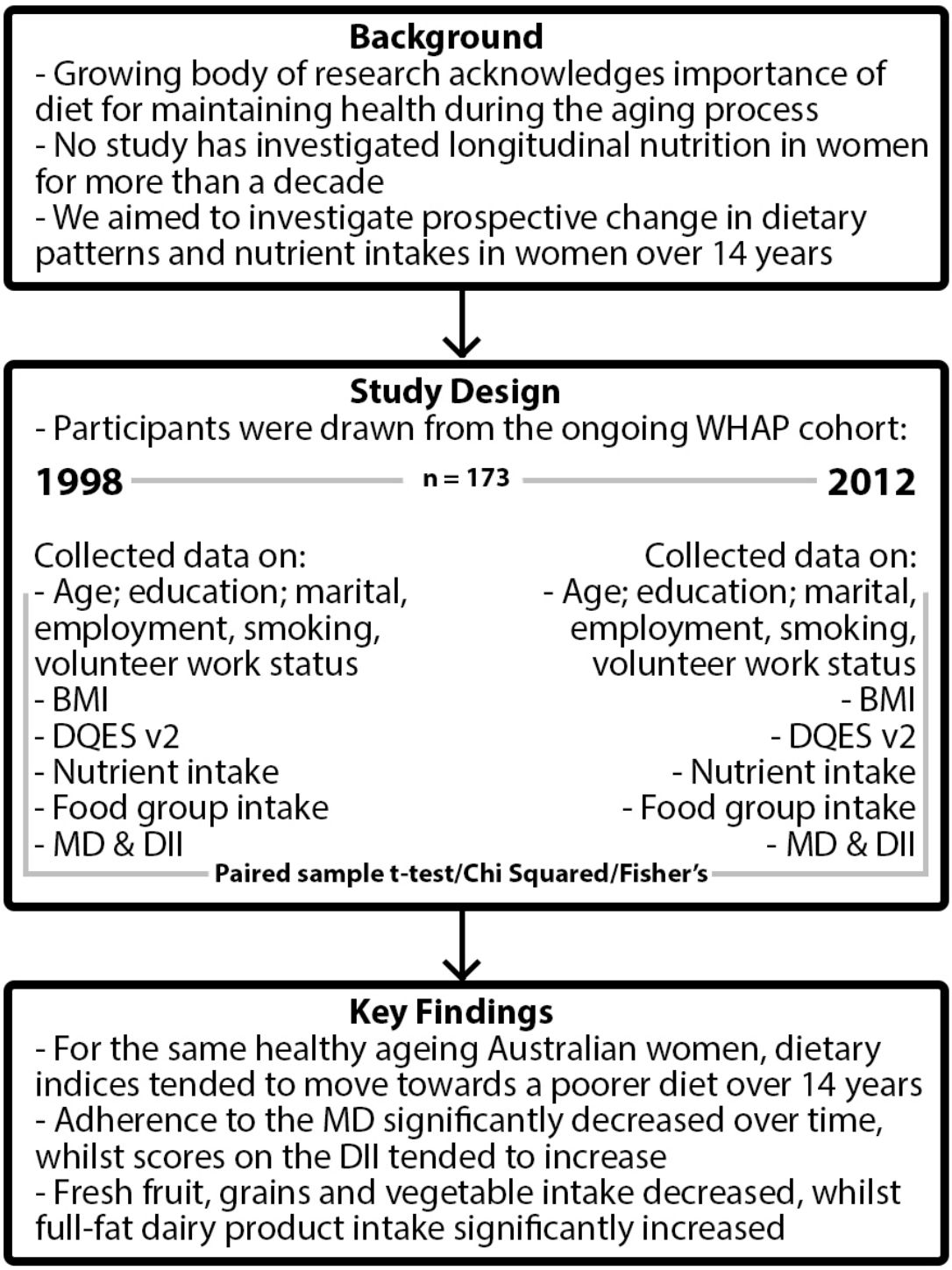

Figure 26: Conceptual diagram of background, study design and key findings

4.3 Results

Sociodemographic characteristics of participants for whom all data were available from both time points are presented in Table 20. The proportion of married participants declined between 1998 (76.3%) and 2012 (61.8%) whilst the proportion of divorced (10.4% in 1998 and 13.9% in 2012) and widowed (4.6% in 1998 and 14.0 % in 2012) participants increased. The number of overweight individuals in the study remained stable (39.3% in 1998 and 39.9% in 2012). The percentage of healthy weight decreased (37.0% in 1998 and 30.1% in 2012) whilst the proportion of individuals categorized as obese increased (23.7% in 1998 and 30.1% in 2012). Overall, 63% of the participants were either overweight or obese in 1998, compared to 70% in 2012. The proportion of smokers decreased over time (13.3% in 1998 and 8.1% in 2012). There was a dramatic decrease in the proportion of participants in paid employment (70.5% in 1998 and 23.1% in 2012); however, the percentage of participants in volunteer work remained stable (55.5% in 1998 and 54.9% in 2012).

Energy-adjusted means and confidence intervals for nutrient intakes, DII and MD adherence are presented in Table 22. Total energy intake decreased over time ($p<0.005$). Percentage of total daily energy intake from the macronutrients total fat, saturated fat, and monounsaturated fat significantly increased while the contribution from carbohydrate significantly decreased over time. Cholesterol displayed a significant increase in intake over time ($p<0.001$). A non-significant trend was observed for an increase in the energy-adjusted intakes of riboflavin, retinol, magnesium and red wine, while a decreasing non-significant trend was evident for vitamin C and sodium ($p<0.02$). MD adherence significantly decreased ($p<0.001$) over time, whilst DII scores displayed a non-significant trend ($p=0.15$) towards a pro-inflammatory diet over time.

Absolute energy and nutrient intakes are provided in Table 24. Carbohydrate (p<0.001), vitamin C (p<0.001), sodium (p=0.001) and α-linolenic acid n-3 (p<0.001) significantly decreased over time. A non-significant trend was observed for an increase in the absolute intakes of cholesterol and red wine (p<0.02), while a decreasing but non-significant trend was observed in the absolute intakes of polyunsaturated fat, fiber, protein, thiamin, niacin, iron, zinc, light beer and spirits (p<0.02).

Nutrients	1998	1998 CI	2012	2012 CI	P-value
Energy (kJ/day)	5772.56	5438.25-6106.86	5322.81	5072.14-5573.48	0.004**
All Fat (g/day)	52.85	48.89-56.82	51.54	48.65-54.43	0.471
Saturated Fat (g/day	20.17	18.52-21.83	20.72	19.38-22.06	0.488
Monounsaturated Fat (g/day)	18.49	16.96-20.03	17.95	16.94-18.97	0.439
Polyunsaturated Fat (g/day)	9.37	8.59-10.15	8.37	7.74-90	0.016
Fiber (g/day)	18.69	17.64-19.74	16.92	15.97-17.86	0.002**
Carbohydrate (g/day)	159.85	151.31-168.39	140.02	132.96-147.09	<0.001***
Protein (g/day)	67.19	63.1-71.28	63.07	60.16-65.99	0.022
Cholesterol (mg/day)	188.73	174.3-203.17	203.46	191.39-215.53	0.021
Beta Carotene (ug/day)	1997.37	1839.9-2154.83	1920.86	1802.38-2039.34	0.372
Folate (mg/day)	228.79	216.08-241.51	218.5	207.28-229.73	0.125
Thiamin (mg/day)	1.25	1.17-1.34	1.14	1.08-1.21	0.011
Niacin (mg/day)	15.80	14.61-16.99	14.13	13.33-14.92	0.005*
Niacin Equivalent (mg/day)	28.84	26.94-30.75	26.46	25.16-27.76	0.008
Riboflavin (mg/day)	1.96	1.83-2.09	1.94	1.83-2.04	0.696
Vitamin C (mg/day	106.63	99.57-113.69	90.19	84.30-96.08	<0.001***
Vitamin E (mg/day)	5.20	4.88-5.52	5.11	4.82-5.40	0.556
Retinol (ug/day)	266.80	247.65-285.95	269.45	251.16-287.74	0.781
Retinol Equivalent (ug/day)	600.5	564.56-636.44	590.40	561.09-619.71	0.579
Iron (mg/day)	9.98	9.34-10.62	9.38	8.83-9.92	0.092
Zinc (mg/day)	8.64	8.12-9.16	8.11	7.73-8.49	0.032
Magnesium (mg/day)	238.82	226.54-251.10	230.71	219.42-241.99	0.195
Sodium (mg/day)	1856.25	1721.78-1990.72	1637.40	1553.98-1720.82	0.001**
α-linolenic acid n-3(g/day)	0.81	0.74-0.88	0.69	0.65-0.73	<0.001***

Long chain n-3 (g/day)	0.33	0.26-0.40	0.31	0.27-0.35	0.580
Omega n-6 (g/day)	7.69	7.04-8.34	7.13	6.57-7.69	0.121
Alcohol (g/day)	14.5	12.16-16.83	15.35	12.6-18.09	0.482
Heavy beer (g/day)	6.37	-2.79-15.53	5.94	-2.68-14.56	0.547
Light beer (g/day)	15.14	3.04-27.25	5.74	0.16-11.32	0.061
Red wine (g/day)	51.15	37.73-64.57	75.36	53.29-97.42	0.020
Spirits (g/day)	4.64	2.93-6.35	2.66	1.61-3.70	0.016
White wine (g/day)	75.12	57.65-92.60	73.45	54.39-92.52	0.862
Garlic (g/day)	0.47	0.41-0.54	0.50	0.43-0.57	0.451
Onion (g/day)	4.56	4.02-5.10	4.15	3.63-4.66	0.139

*p <0.01, **p<0.005, ***p<0.001. Daily intakes expressed

Table 24: Absolute daily intakes and 95% CIs for intakes of WHAP participants by year of dietary survey

Estimated energy-adjusted daily intakes in 1998 and 2012 of 33 *a priori* defined food groups are provided in Table 23. Fresh fruit (p<0.001), refined grains (p<0.001), red meats (p<0.02), poultry (p<0.02), fruit juice (p<0.001), added sugar (p<0.001), unsaturated spreads (p=0.02) and alcoholic spirits (p<0.001) significantly decreased over time. Full-fat dairy products (p<0.01), nuts (p<0.001) and eggs (p<0.001) all significantly increased.

The associations of changes in employment status (paid/volunteer), marital status and BMI with diet were assessed. The majority of participants who transitioned out of paid employment over the 14 years showed a decrease in MD adherence. Only 2 participants transitioned into paid employment and they also displayed a decreased MD (p=0.19). Taking on volunteer work tended to be associated with increased MD adherence, whilst giving up volunteer work tended to be associated with a decreased MD score (p=0.13). Those who became widowed over the 14 years tended to decrease MD adherence,

whilst those who married tended to increase their MD score (p=0.16). There were no relationships observed between change in MD adherence and age, education, smoking status or BMI.

4.4 Discussion

This study shows significant changes in the intake of several nutrients and MD adherence in a cohort of aging Australian women in the Melbourne metropolitan area over a period of 14 years. Energy intake decreased over time as might be expected with aging and some absolute and energy-adjusted nutrients and macronutrients showed significant changes. Scores on both the DII and the MD adherence showed change towards poorer diet quality between the two time points; DII values tended to increase, becoming more pro-inflammatory over time, although they remained on the anti-inflammatory side of the scale and the observed change was not significant.

Our results, showing a decrease in total energy intake over 14 years, is in line with a British study in adults followed over 17 years that found a decrease in energy consumption in men and women between the ages of 43 and 53 years, although energy intake had increased between ages 36 and 43 years [328]. These changes observed in the British study may reflect changes in food choices as well as well as the amount of food consumed. Prynne*, et al.* [328] also found fat intake, as a percentage of energy, decreased over time; however, the current study found significant increases in the contribution of fat to energy intake in Australian women from mean age 55 years to 70 years. In the current study there was a non-significant trend for vitamin C intake to decrease and vitamin E intake to increase, however, Prynne*, et al.* [328] found energy-adjusted increases for both reflecting an overall change to a diet more closely reflecting dietary guidelines.

As a percentage of total energy and as an absolute value, carbohydrate intake significantly decreased over time in the WHAP participants. This is in contrast to the

findings of Prynne*, et al.* [328] where women's total carbohydrate and percentage of energy from carbohydrate intake gradually increased over a period of 17 years (aged 36 to 53 years). Given the overall trend in the birth cohort studied by Prynne*, et al.* [328] towards a more healthy diet over time, it is possible the increase in carbohydrate intake was in favour of more complex carbohydrates, although this is not noted.

The current study found that the proportion of energy contributed by total fat, saturated fat and monounsaturated fat all increased significantly over 14 years, as did the intake of cholesterol relative to energy, consistent with increased consumption of animal-based foods. The small increase in riboflavin intake over time and the significant increase of full-fat dairy products also suggests an increase in the relative consumption of milk and milk products, one of the main sources of riboflavin in the Australian diet [329].

The changes reported are in line with a transition towards a less healthy diet and are reflected in the BMI shifting towards obesity in the context of declining physical activity and reduced energy requirements with age [330]. We also observed widowhood becoming more prevalent over 14 years, with participants who were widowed more likely to decrease MD adherence compared with participants experiencing a stable marital situation. Widowhood can have a dramatic impact on the surviving spouse, catalyzing changes in daily routines and dietary choices that may adversely affect their nutritional status [298]. Widowed individuals are more likely to consume non-nutritious foods, fewer vegetables and are less likely to prepare homemade meals than their married peers [298,299,331,332]. The loss of a spouse also is related to a decrease in dietary diversity [331,332], reflecting a deprioritization of personal nutrition. In the WHAP cohort, becoming a widow was associated with a transition towards a less healthy dietary pattern, consistent with a reorientation of cooking habits and food behaviors. Conversely, women who married during the period were more likely to display an increase in MD adherence. Individuals who marry in later life are likely to reprioritize

personal nutrition to coincide with changes in their social environment and may display healthier dietary patterns than their unmarried peers [331,333]. We observed an increased MD adherence for those women taking up voluntary work when compared with those giving up volunteer work over the 14 years. Volunteer responsibilities can catalyse social engagement and offer opportunities for the dissemination of healthy eating habits.

A growing body of research is acknowledging the importance of the MD categories in nutrition research [169,334-336]. Of the food groups that made up the MD score; fruit, cereal, meat and vegetable intake decreased; legumes and fish remained constant; whilst intake of dairy products and wine increased. MD adherence and non-significant increases in DII scores both indicated a transition towards a less healthy diet over time. This is in contrast to several longitudinal dietary studies that have found stability for healthier eating patterns in women [321,337] and in unhealthy Western patterns in men [338]. In a 10-year follow up of individuals aged 50-69 years at baseline, latent class analysis revealed women were significantly more likely to be in the healthy stable group and men in the Western stable group [337]. Consumption of fruit and vegetables increased over time and this increase was greater in women, indicating they may be more responsive to health promotion messages [293]. Compared to Prynne*, et al.* [328], who reported a trend towards a better diet over time, the current study observed a trend towards poorer diet quality and this may relate to the older age of women in our cohort. In the WHAP cohort, we observed consumption of fresh fruit and refined grains significantly decreased over time and several vegetable groups displayed non-significant decreases over 14 years. Given similar changes were observed for Anglo-Celt and Greek-Australian men and women aged above 70 years[323], we hypothesise the differences between WHAP and the UK cohort studied by Prynne*, et al.* [328] may be age-related. Aged between 36 and 53 years, the UK cohort[328] was at lower risk for the loss of employment, physical function, widowhood and social engagement than would be expected of women aged between 55

and 69 years. Compared to other studies such as Shivappa*, et al.* [276], scores and range of the DII values were low. DII scores were in a similar range to those observed by Tabung [324] in a cohort of postmenopausal women.

One of the strengths of this study was that the FFQ and method of nutrient calculation were identical for both time points; therefore, reported changes in nutrient intakes are largely due to changes in portion size or frequency of consumption and will not be confused by changes in the dietary methodology. However, as has been previously discussed [328], our analysis is not able to account for changes in the composition of individual food items by manufacturers over time. Short-term follow-up dietary studies often assume dietary stability without capturing long term change therefore our study was strengthened by 14 years of follow-up dietary data on the same women. Of the 438 WHAP participants who underwent assessments in 1998, 173 who also participated in the 2012 survey were included in this analysis. There were no significant differences in education, employment, age, BMI, smoking status, nutrient intake or MD adherence between the excluded and included participants in this study. This suggests that the loss to follow-up has not introduced bias into this investigation.

A limitation of this study was the lack of socioeconomic status as a covariate and the small sample size; however, this study is part of a longitudinal investigation over 25 years and drop out over this period of time is inevitable.

In conclusion, small changes in intakes of a range of dietary components resulted in changes towards poorer diet quality as assessed by the MD score and, though not statistically significant, the DII in these women as they aged. Given the importance of maintaining a healthy diet in supporting health and function with aging, this is of concern and requires further study to identify factors associated with worsening dietary patterns and thus facilitate targeting dietary interventions to women who need it most.

5 LONGITUDINAL DIETARY PATTERNS

This chapter presents an investigation of prospective changes in dietary changes in participants of the WHAP between 1998 and 2012. Factor analysis was utilised on WHAP participants at both time points to identify two 'healthy-type' patterns and another less healthy pattern. This chapter elaborates on how some women's dietary patterns remained stable, the majority underwent dietary change at this stage of their lives.

This chapter contains a modified version of the following publication:

Hill E, Hodge A, Gorelik A, Shang X, Dennerstein L, Szoeke C Longitudinal dietary patterns in ageing Australian women: Data from the Women's Healthy Ageing Project. Asia Pacific Journal of Clinical Nutrition (Under Review)

5.1 Introduction

The world's population is ageing. Since 1980, the global population aged over 60 years has increased from 382 million to 962 million in 2017 [340]. This number is projected to double again by 2050, when the number of individuals aged over 60 years worldwide will reach nearly 2.1 billion [340]. The ageing population will place significant demands on healthcare, support and civil services [341], motivating research into modifiable lifestyle factors that may promote healthy ageing.

An increasing body of research acknowledges the importance of dietary patterns in healthy ageing [286]. During the ageing process there is a natural decrease in caloric intake [342], heightening the importance of investigating dietary sufficiency in ageing adults. Diet is a key determinant of chronic disease in later life and an integral component of successful ageing [343]. Dietary patterns are easily monitored via food diaries, food frequency questionnaires and surveys. Patterns can be linked to dietary advice that can mitigate weight gain or loss [344] leading to considerable health benefits [345,346]. Adopting and maintaining healthy dietary habits may contribute to minimising disease risk, maintaining functional independence and therefore maximising quality of life and independence throughout adulthood to later life [347].

In research, diet and health outcomes can be represented in a variety of methods. Food and nutrient intake can be assessed either as individual continuous variables, or relative adherence to recommended intake. However, dietary pattern scores or indices assess the whole diet. Diet pattern analysis is primarily conducted using two types of method: *a priori* methods assess how well a diet matches with a pre-determined or 'ideal' diet; data-driven or *a posteriori* methods use statistical techniques to identify patterns of intake from within the study population [255]. *A posteriori* dietary patterns have attracted

substantial research interest as they summarise the large variations in dietary intake into a distilled set of factors, enabling the subjective characterisation of the diet [307]. Nutritional epidemiological research has identified recurring patterns in different populations: "healthy" dietary patterns are primarily made up of vegetables, fruits, fish and low-fat foods; whereas "unhealthy" patterns tend to include meat, high-fat, sugar-rich or fried foods [348,349].

Although dietary intake varies substantially throughout life, epidemiological research often measures diet at a single time-point under the assumption of longitudinal dietary stability. Currently few studies have investigated dietary patterns longitudinally in ageing individuals. Over 17 years of follow up in a British population study (1946 British Birth Cohort), a factor analysis by Mishra*, et al.* [321] found key differences in dietary patterns by sex. Mishra*, et al.* [321] identified three dietary patterns in women ("fruit, vegetables and dairy"; "ethnic foods and alcohol"; "meat, potatoes and sweet foods") and two patterns in men ("ethnic foods and alcohol"; "mixed"). This involved dichotomising food into eaten/not eaten and conducting exploratory factor analysis on the diets of participants at the last time point (age = 53 years) then cross-checked to determine dietary stability at the two previous time points (age = 36 years and 43 years). Moderate stability was reported in two dietary patterns ("fruit, vegetables and dairy" and "mixed"); however the "meat, potatoes and sweet foods", identified only in women, showed poor stability over time. In a population study across Switzerland, Marques-Vidal*, et al.* [350] characterised dietary patterns over 20 years of follow up utilising principal component analysis on all the available data. Investigating different people at each time point, Marques-Vidal*, et al.* [350] explored generational trends, reflecting population dietary patterns rather than individual change. Three dietary patterns emerged, two were considered unhealthy ("meat and chips", "chocolate and sweets") and one healthy ("fish and vegetables"). Scores on the "fish and vegetables" pattern

increased, whereas the "meat and chips" and "chocolate and sweets" pattern decreased over time. Population trends were also estimated from participants in the Swedish Mammography Cohort by Newby*, et al.* [351] in exploratory and confirmatory factor analysis on food frequency questionnaires ten years apart (1987 and 1997). Newby*, et al.* [351] observed four food patterns ("Healthy", "Western/Swedish", "Alcohol" and "Sweets") and analysed correlation coefficients to suggest these patterns were largely stable in different individuals over ten years. Song*, et al.* [352] combined longitudinal data in a factor analysis to classify three dietary patterns ("traditional", "modified" and "western") in a Korean population. Over 7 years of follow up, Song*, et al.* [352] observed trends towards a decrease in adherence to the "traditional" dietary pattern and a gradual increase in proportions of the "modified" and "western" patterns. In a population study employing exploratory factor analysis at each of 7 surveys in China, Batis*, et al.* [353] found adherence to two key dietary patterns remained largely stable over 18 years. Congruence coefficients were assessed to illustrate the structure of the dietary patterns and the individuals adhering to them remained reasonably constant over follow-up. The traditional "southern" pattern was characterised by high intakes of rice, fresh leafy vegetables, low-fat red meat, pork, organ meats, poultry and fish/seafood and low intakes of wheat flour/maize/coarse grains. The "modern" dietary pattern was characterised by high intakes of wheat buns/breads, cakes/cookies/pastries, deep-fried wheat, nuts/seeds, starchy root/tuber products, fruits, eggs/egg products, soya milk, animal-based milk and instant noodles/frozen dumplings.

Studies that investigate the changing dietary habits of a population are important to monitor diet-disease associations, identify risk reduction methods and to adapt and promote the adoption of a healthy dietary pattern. To maintain successful health in ageing, research is required to define healthy dietary patterns; however, only one study has investigated changing dietary patterns in the same women over time. We previously

reported on significant nutritional changes toward an unhealthy dietary pattern [354]; however, to date, no study has investigated longitudinal dietary patterns in ageing Australian women. We aimed to identify and evaluate the stability of dietary patterns over time in ageing Australian women.

5.2 Methods

5.2.1 Participants

Participants included in this study were from the 1998 and 2012 follow-up of the Women's Health Ageing Project (WHAP), an epidemiologically sourced prospective study of healthy aging Australian women. WHAP is an extension of the Melbourne Women's Midlife Health Project. Briefly, 438 women within the Melbourne metropolitan area were identified by random digit dialling in 1991. Women were eligible for the cohort if they were Australian-born, aged 45–55 years, had menstruated in the three months before recruitment, and were not taking estrogen-containing hormone replacement therapy. Longitudinal follow-up was completed annually until 1999 and intermittently thereafter. In 2012, participants were re-contacted and invited to participate in a late-life health study. Clinical assessments were conducted on 252 participants by trained field researchers. The clinical assessments included a battery of validated measures of physical health, sociodemographics, lifestyle, cognitive function, psychological health, and biomarkers. A complete methodology has been published elsewhere [355].

5.2.2 Dietary assessment

Participants completed a validated food frequency questionnaire in both 1998 and 2012; the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2) [272]. The DQES v2 incorporates 80 food items and covers five broad types of dietary intake: cereals, sweets and snacks; dairy, meat and fish; fruits; vegetables; and alcoholic

beverages. Data collected by the DQES v2 was used to calculate daily energy and nutrient intakes by the Cancer Council of Victoria based on Australian nutrient composition data from NUTTAB95[356] and collated via the Composition of Foods, Australia [273]. Individuals were excluded from this analysis if their energy intake was calculated as less than 3000 kJ/day, or over 20,000 kJ/day. All food items were reported in grams per day and classified into 33 food groups defined *a priori* (Table 25) that were similar to those used by others [283,284].

Food Group	Items in the DQES v2
Whole grains	All bran, bran flakes, high fibre white bread, muesli, multigrain bread, porridge, rye bread, Weet-Bix, wholemeal bread
Refined grains	Corn flakes, crackers, pasta, rice, white bread
Red meats	Beef, lamb, pork, veal
Processed meats	Bacon, salami, sausages
Poultry	Chicken
Takeaway foods	Hamburger, meat pies, pizza
Fried fish	Fried fish
Other fish	Fish (non-fried), tinned fish
Fried potatoes	Chips (French fries)
Other potato	Potatoes
Yellow or red vegetables	Capsicum, carrots, pumpkin
Legumes	Baked beans, green beans, other beans, peas, tofu
Cruciferous vegetables	Broccoli, cabbage, cauliflower
Leafy green vegetables	Lettuce, spinach
Other vegetables	Bean sprouts, beetroot, celery, cucumber, garlic, mushrooms, onion, zucchini
Tomato	Tomatoes
Fresh fruit	Apples, apricots, avocado, bananas, mango, melon, oranges, peaches, pears, pineapple, strawberries
Canned fruit	Tinned fruit
Cakes, biscuits, sweet pastries	Cakes, sweet biscuits
Low-fat dairy products	Flavoured milk drink, low-fat cheese, reduced fat milk, ricotta cheese, cottage cheese, skim milk
Full- fat dairy products	Cream cheese, firm cheese, full-cream milk, hard cheese, ice cream, soft cheese, yoghurt
Soya milk	Soya milk
Confectionery	Chocolate

Added sugar	Jam, sugar
Crisps	Crisps
Nuts	Nuts, peanut butter
Eggs	Eggs
Fruit juice	Fruit juice
Saturated spreads	Butter, butter-margarine blends, margarine
Unsaturated spreads	Monounsaturated margarine, polyunsaturated margarine
Alcohol - beer	Heavy beer, light beer
Alcohol - wine	Red wine, white wine
Alcohol - spirits	Fortified wines, spirits

Table 25: Food groupings and items within the Dietary Questionnaire for Epidemiological Studies Version 2

5.2.3 Statistical analysis

All statistics were produced in STATA software on Windows operating system. Complete dietary data was available for 183 WHAP participants and there were no statistically significant differences between the included (1998: n = 183, 2012: n = 183) and excluded women (1998: n = 199, 2012: n = 63). Unadjusted and energy adjusted mean daily DQES v2 food group intakes are displayed in, respectively, Table 26 and Table 27. Dietary intake was adjusted by dividing the intakes of individual food items (n = 80) by a participant's estimated daily energy intake (MJ/day). Factor analysis of energy adjusted intakes of food items grouped into the categories defined *a priori* was conducted using iterated principal factor analysis (IPFA) with oblique varimax rotation due to the presumed intercollinearity and nonindependence of dietary patterns. Dietary factors to retain were identified using criteria described by others [357,358]: an eigenvalue >1; scree plot analysis and interpretability. Food/beverage items were considered characteristic of a dietary pattern if their loadings were of greater magnitude than or equal to 0.2 or -0.2.

Food Group	1998 Mean	1998 CI	2012 Mean	2012 CI	p values
Energy (kJ/day)	5813.1	5488.7 - 6137.4	5322.8	5082.3 - 5563.4	**<0.001*****
Whole grains (g/day)	109.1	98.1 - 120.1	102.7	92.6 - 112.8	0.262
Refined grains (g/day)	88.1	74.5 - 101.7	51.4	44.1 - 58.8	**<0.001*****
Red meats (g/day)	40.4	36.3 - 44.6	34.9	30.9 - 38.8	0.010
Processed meats (g/day)	10.4	5.9 - 14.9	7.4	6.0 - 8.8	0.105
Poultry (g/day)	19.7	16.1 - 23.4	14.9	13.4 - 16.5	0.008*
Takeaway foods (g/day)	25.0	21.7 - 28.3	24.0	19.4 - 28.6	0.714
Fried fish (g/day)	3.3	2.2 - 4.4	3.0	2.2 - 3.7	0.571
Other fish (g/day)	23.1	18.4 - 27.8	22.3	18.6 - 25.9	0.723
Fried potatoes (g/day)	7.6	6.3 - 8.9	8.8	7.3 - 10.4	0.161
Other potato (g/day)	28.4	25.1 - 31.7	24.5	21.0 – 28.0	0.060
Yellow or red vegetables (g/day)	22.6	20.6 - 24.7	22.4	20.3 - 24.5	0.851
Legumes (g/day)	21.2	19.1 - 23.3	21.9	19.8 – 24.0	0.558
Cruciferous vegetables (g/day)	22.3	20.1 - 24.6	23.2	20.8 - 25.6	0.508
Leafy green vegetables (g/day)	11.7	10.3 - 13.1	12.9	11.8 – 14.0	0.137
Other vegetables (g/day)	24.8	22.7 - 26.9	23.9	22.2 - 25.6	0.422
Tomato (g/day)	11.9	10.3 - 13.4	13.1	11.3 - 14.8	0.284
Fresh fruit (g/day)	220.5	203.9 - 237.2	185.4	169.8 - 201	**<0.001*****
Canned fruit (g/day)	11.0	7.1 - 14.9	10.3	7.4 - 13.2	0.762
Cakes, biscuits, sweet pastries (g/day)	19.2	15.6 - 22.8	17.6	15.1 - 20.2	0.404
Low-fat dairy products (g/day)	216.9	189.9 - 243.9	220.5	192.0 - 248.9	0.811
Full-fat dairy products (g/day)	106.7	89.5 - 123.8	139.0	118.9 - 159.1	0.004*
Soya milk (g/day)	49.3	30.0 - 68.7	35.3	21.0 - 49.7	0.178
Confectionery (g/day)	4.5	3.2 - 5.9	6.3	4.9 - 7.7	0.048
Added sugar (g/day)	11.7	9.7 - 13.7	8.3	6.8 - 9.7	**<0.001*****
Crisps (g/day)	1.8	1.3 - 2.3	1.5	1.0 - 1.9	0.255
Nuts (g/day)	2.9	2.1 - 3.7	4.8	3.8 - 5.8	**<0.001*****
Eggs (g/day)	11.7	10.4 – 13.0	16.3	14.7 - 18	**<0.001*****
Fruit juice (g/day)	50.6	40.5 - 60.7	24.6	18.6 - 30.6	**<0.001*****
Saturated spreads (g/day)	3.7	2.5 - 4.8	2.6	1.8 - 3.5	0.120
Unsaturated spreads (g/day)	8.1	6.5 - 9.6	5.8	4.6 - 7.1	0.009*
Alcohol beverage - beer (g/day)	21.0	6.7 - 35.3	11.7	2.0 - 21.4	0.058

Alcohol beverage - wine (g/day)	130.8	105.9 - 155.7	152.2	124.4 - 179.9	0.099
Alcohol beverage - spirits (g/day)	13.3	9.4 - 17.1	6.9	4 - 9.9	0.003**

CI, Confidence Intervals (95%). *p <0.01, **p<0.005, ***p<0.001. Daily intakes expressed.

Table 26: Mean daily intakes by DQES v2 category

Food Group	1998 Mean	1998 CI	2012 Mean	2012 CI	p values
Energy (kJ/day)	5813.1	5488.7 - 6137.4	5322.8	5082.3 - 5563.4	**<0.001*****
Whole grains (g/MJ/day)	19.3	17.6 - 21	18.9	17.4 - 20.5	0.713
Refined grains (g/MJ/day)	14.1	12.8 - 15.4	9.4	8.4 - 10.5	**<0.001*****
Red meats (g/MJ/day)	7.0	6.4 - 7.6	6.5	5.8 - 7.1	0.137
Processed meats (g/MJ/day)	1.5	1.2 - 1.7	1.4	1.2 - 1.6	0.716
Poultry (g/MJ/day)	3.3	3.0 - 3.7	2.9	2.6 - 3.2	0.016
Takeaway foods (g/MJ/day)	4.3	3.8 - 4.7	4.4	3.7 - 5.1	0.753
Fried fish (g/MJ/day)	0.6	0.4 - 0.7	0.5	0.4 - 0.7	0.880
Other fish (g/MJ/day)	3.8	3.2 - 4.3	4.1	3.6 - 4.6	0.318
Fried potatoes (g/MJ/day)	1.3	1.1 - 1.6	1.6	1.4 - 1.9	0.068
Other potato (g/MJ/day)	5.1	4.5 - 5.7	4.6	4.0 - 5.3	0.256
Yellow or red vegetables (g/MJ/day)	4.1	3.7 - 4.4	4.6	3.9 - 5.4	0.137
Legumes (g/MJ/day)	3.8	3.4 - 4.2	4.3	3.9 - 4.7	0.040
Cruciferous vegetables (g/MJ/day)	4.2	3.7 - 4.6	4.8	4.2 - 5.4	0.031
Leafy green vegetables (g/MJ/day)	2.1	1.9 - 2.4	2.6	2.4 - 2.9	0.003**
Other vegetables (g/MJ/day)	4.5	4.1 - 4.9	4.7	4.4 - 5.1	0.365
Tomato (g/MJ/day)	2.1	1.8 - 2.4	2.7	2.3 - 3.1	0.018
Fresh fruit (g/MJ/day)	40.1	36.9 - 43.3	35.8	32.9 - 38.7	0.016
Canned fruit (g/MJ/day)	1.8	1.2 - 2.4	1.9	1.4 - 2.4	0.896
Cakes, biscuits, sweet pastries (g/MJ/day)	3.1	2.6 - 3.6	3.1	2.7 - 3.5	0.810
Low-fat dairy products (g/MJ/day)	41.2	35.8 - 46.6	45.6	38.4 - 52.8	0.246
Full-fat dairy products (g/MJ/day)	18.7	15.7 - 21.6	26.3	22.5 - 30.1	**<0.001*****
Soya milk (g/MJ/day)	9.0	4.5 - 13.4	7.4	3.8 - 10.9	0.558
Confectionery (g/MJ/day)	0.7	0.5 - 0.8	1.1	0.9 - 1.3	0.001**
Added sugar (g/MJ/day)	1.9	1.6 - 2.2	1.5	1.2 - 1.7	0.003**
Crisps (g/MJ/day)	0.3	0.2 - 0.4	0.3	0.2 - 0.4	0.664
Nuts (g/MJ/day)	0.4	0.3 - 0.4	0.7	0.6 - 0.8	**<0.001*****
Eggs (g/MJ/day)	2.1	1.9 - 2.3	3.2	2.9 - 3.5	**<0.001*****
Fruit juice (g/MJ/day)	8.9	7.2 - 10.5	4.7	3.5 - 5.9	**<0.001*****
Saturated spreads (g/MJ/day)	0.6	0.4 - 0.8	0.5	0.4 - 0.7	0.393
Unsaturated spreads (g/MJ/day)	1.4	1.1 - 1.7	1.1	0.9 - 1.3	0.034
Alcohol beverage - beer (g/MJ/day)	3.7	1.1 - 6.3	2.4	0.2 - 4.7	0.035
Alcohol beverage - wine (g/MJ/day)	25.6	20.6 - 30.7	32.0	25 - 38.9	0.023
Alcohol beverage - spirits (g/MJ/day)	2.5	1.8 - 3.2	1.5	0.8 - 2.1	0.007*

CI, Confidence Intervals (95%). *p <0.01, **p<0.005, ***p<0.001. Daily intakes expressed.

Table 27: Energy adjusted mean daily intakes by DQES v2 category

Adherence to identified dietary patterns was converted from the highest loading dietary pattern factor score to binary pattern adherence to minimise the intra-correlations between variables. Unadjusted mean DQES v2 daily intakes grouped by identified dietary pattern are displayed in Table 28. Paired sample t-tests were conducted to evaluate individual differences in unadjusted energy and food group intakes between 1998 and 2012. Multiple analyses were adjusted for using the Bonferonni method. Population characteristics according to highest scoring dietary patterns were presented, including age in years, education in years, body mass index (BMI), energy intake (kJ/day), marital status (single, married, divorced, separated, widowed or other) and smoking status (current smoker or current non-smoker).

	1998			**2012**		
	Fruit & Veg	**Meat & Potato**	**Meat & Low-Fat Dairy**	**Fruit & Veg**	**Meat & Potato**	**Veg & Soy Milk**
	n = 63	**n = 64**	**n = 56**	**n = 64**	**n = 73**	**n = 46**
Energy (kJ/day)	5179.2 ± 217.5	6530.2 ± 358.6	5706.6 ± 211.5	4825.4 ± 203.4	5792.6 ± 193.8	5269.5 ± 218.3
Whole grains (g/day)	121.3 ± 9.6	63.7 ± 5.2	147.2 ± 10.5	103.7 ± 7	84.8 ± 8.6	129.6 ± 10.5
Refined grains (g/day)	76.5 ± 7.4	119.8 ± 16.8	65.1 ± 6.4	40.8 ± 3.4	63 ± 5.3	47.9 ± 11
Red meats (g/day)	27.5 ± 2.7	51.6 ± 4.2	42.1 ± 3.1	22.5 ± 2	49.9 ± 3.7	28.2 ± 2.8
Processed meats (g/day)	4.9 ± 0.8	19 ± 6.3	6.8 ± 0.6	5.3 ± 0.5	11.2 ± 1.6	4.3 ± 0.7
Poultry (g/day)	16.9 ± 1.6	23.9 ± 4.9	18.2 ± 1.6	11.7 ± 1	19.4 ± 1.4	12.3 ± 1.4
Takeaway foods (g/day)	17.2 ± 1.6	37.1 ± 3.8	20 ± 1.9	17.4 ± 1.8	32.7 ± 4.9	19.3 ± 4.2
Fried fish (g/day)	1.2 ± 0.3	4 ± 0.9	4.9 ± 1.4	1.6 ± 0.4	4.7 ± 0.8	2 ± 0.4
Other fish (g/day)	21.4 ± 3	24 ± 4.5	23.9 ± 4.7	22.5 ± 2.3	23.4 ± 4	20.1 ± 2.1
Fried potatoes (g/day)	3.7 ± 0.5	11.9 ± 1.4	7.2 ± 1	4.7 ± 0.7	14.6 ± 1.6	5.5 ± 0.8
Other potato (g/day)	26.3 ± 2.6	29.2 ± 3.2	29.8 ± 2.9	18.8 ± 2	27.8 ± 3.3	27 ± 3.6
Yellow or red vegetables (g/day)	28.9 ± 2.1	18 ± 1.2	20.9 ± 1.6	19.2 ± 1.1	20.9 ± 1.6	29.1 ± 2.7
Legumes (g/day)	24 ± 2.3	21.8 ± 1.6	17.4 ± 1.4	22.4 ± 1.7	20.7 ± 1.8	23.2 ± 2.1
Cruciferous vegetables (g/day)	27.9 ± 2.2	18 ± 1.5	21 ± 1.8	20.5 ± 1.7	18.4 ± 1.6	34.5 ± 2.7
Leafy green vegetables (g/day)	15.7 ± 1.6	8.8 ± 0.8	10.4 ± 0.7	17.5 ± 0.9	10.9 ± 0.8	9.7 ± 1

Other vegetables (g/day)	32.5 ± 2.1	18.8 ± 1.4	22.9 ± 1.2	28.6 ± 1.3	20.9 ± 1.3	22.2 ± 1.7
Tomato (g/day)	14.8 ± 1.6	9.2 ± 1.2	11.7 ± 1.3	19.3 ± 1.8	9 ± 0.9	10.7 ± 1.6
Fresh fruit (g/day)	266.6 ± 14.6	179.6 ± 11.9	215.5 ± 15.3	222.5 ± 13.4	156.8 ± 11.1	179.1 ± 16.3
Canned fruit (g/day)	10.6 ± 3.2	10.4 ± 2.7	12.1 ± 4.3	6.7 ± 1.6	9.4 ± 1.9	16.6 ± 4.4
Cakes, biscuits, sweet pastries (g/day)	14.9 ± 2.8	30.7 ± 3.9	10.8 ± 1.4	15 ± 2.2	21.3 ± 2.2	15.5 ± 2.2
Low-fat dairy products (g/day)	175.9 ± 21.1	202 ± 26.1	280.1 ± 21.4	203.4 ± 21.5	199.3 ± 21.5	277.7 ± 34.1
Full-fat dairy products (g/day)	120.7 ± 15.6	133.1 ± 17.3	60.6 ± 7.9	124 ± 14.3	155.8 ± 18.8	133.2 ± 18.9
Soya milk (g/day)	63.7 ± 16.3	50.6 ± 18.9	31.7 ± 15	29.9 ± 10.6	23.6 ± 9.8	61.4 ± 19.3
Confectionery (g/day)	1.8 ± 0.3	8.3 ± 1.8	3.2 ± 0.6	6.7 ± 1.5	6.1 ± 0.9	6.1 ± 1.6
Added sugar (g/day)	7.8 ± 1.3	17.3 ± 2.1	9.7 ± 1.6	5.8 ± 1.1	12.1 ± 1.4	5.6 ± 1.1
Crisps (g/day)	1.4 ± 0.3	2.7 ± 0.6	1.3 ± 0.3	0.9 ± 0.2	2.3 ± 0.5	1 ± 0.4
Nuts (g/day)	3.5 ± 0.9	2.3 ± 0.4	3 ± 0.5	4.8 ± 0.8	4 ± 0.6	5.9 ± 1.3
Eggs (g/day)	11.3 ± 1.2	13.2 ± 1.3	10.4 ± 0.8	17.1 ± 1.5	16.9 ± 1.4	14.3 ± 1.5
Fruit juice (g/day)	49.8 ± 7.1	40.8 ± 6.4	62.9 ± 12.7	31.8 ± 6.2	24.8 ± 4.7	14.5 ± 3.7
Saturated spreads (g/day)	3.7 ± 1.1	6.2 ± 1.1	0.8 ± 0.6	2 ± 0.6	1.9 ± 0.6	4.7 ± 1.1
Unsaturated spreads (g/day)	4.6 ± 1.1	5.5 ± 1.1	14.9 ± 1.5	4.6 ± 0.9	7.3 ± 1.2	5.3 ± 1.1
Alcohol beverage - beer (g/day)	18.6 ± 12.7	22.5 ± 11.1	21.9 ± 14.2	16.6 ± 11.8	10.3 ± 6.6	7.1 ± 3.2
Alcohol beverage - wine (g/day)	160.0 ± 26.4	101.3 ± 18.2	131.6 ± 19.1	191.9 ± 30.2	143.8 ± 18.9	110.1 ± 20.3
Alcohol beverage - spirits (g/day)	8.0 ± 1.7	14.9 ± 4	17.4 ± 4	6.5 ± 1.6	8.9 ± 3.3	4.3 ± 1.3

± Indicates standard deviation

Table 28: Unadjusted mean DQES v2 daily intakes by dietary pattern identified by IPFA

5.3 Results

Participants mean age was 55.6 (± 2.4) years in 1998 and 69.4 (± 2.6) years in 2012, with an average of 12.6 (±3.5) years of (Table 29). The percentage of WHAP participants who reported being married (77.6% in 1998 to 61.5% in 2012) declined, as with those who reported being single (3.3% in 1998 to 2.8% in 2012). Proportions increased for those who were divorced (9.8% in 1998 to 13.2% in 2012), separated (2.7% in 1998 to 3.3% in 2012) or widowed (4.4% in 1998 to 14.8% in 2012). The proportion of participants in the healthy BMI range (>= 18.5 and < 25kg/m2) decreased

from 36.1% in 1998 to 30.3% in 2012. Most WHAP participants (69.7%) were either overweight (>=25 and <30kg/m2) or obese (>=30kg/m2) by 2012 and were non-smokers (86.3% in 1998 to 92.3% in 2012).

		1998	2012
Age			
	Mean age in years	55.6 ± 2.4	69.9 ± 2.6
Marital Status			
	Single	6 (3.3%)	5 (2.8%)
	Married	142 (77.6%)	112 (61.5%)
	Divorced	18 (9.8%)	24 (13.2%)
	Separated	5 (2.7%)	6 (3.3%)
	Widowed	8 (4.4%)	27 (14.8%)
	Other	4 (2.2%)	8 (4.4%)
Education		**Education reported in 2012**	
	Education (including primary school) in years	12.6	
BMI category			
	Healthy weight (>= 18.5 and < 25kg/m^2)	66 (36.1%)	54 (30.3%)
	Overweight (>=25 and <30kg/m^2)	69 (37.7%)	71 (39.9%)
	Obese (>=30kg/m^2)	42 (23.0%)	53 (29.8%)
Smoking status			
	Current smoker	25 (13.7%)	14 (7.7%)
	Current non-smoker	158 (86.3%)	167 (92.3%)

BMI, body mass index. Expressed are numbers (%). Percentage is of time point population total. Underweight (18.5kg/m2) removed due to no WHAP participant fitting in this category at either time point.

Table 29: Characteristics of WHAP study population in 1998 and 2012 (n = 183)

Three dietary patterns were identified for both 1998 and 2012 and were characterised according to their respective factor loadings in Table 30. A “Fruit & Veg” pattern was characterised by a high intake of fruit (fresh fruit, tomato) and vegetables (yellow or red vegetables, other vegetables, legumes, leafy green vegetables, cruciferous vegetables and other potato) was identified and 63 women were classified to this. The “Fruit & Veg” dietary pattern was also highly inversely loaded on confectionary and added sugar products. Sixty four participants were found to adhere to a “Meat & Potato” dietary pattern, characterised by a high intake of meats (processed meats, red meats, poultry,

refined grains and takeaway foods) and fried potatoes. The “Meat & Potato” pattern inversely loaded on whole grains, fresh fruit and soy milk. The third pattern identified in 1998 was a “Meat & Low-Fat Dairy” pattern, characterised by a high intake of red meat, poultry, non-fried potatoes, low-fat dairy products and unsaturated spreads, with inverse loadings for cakes, biscuits, pastries, added sugar and saturated spreads with 56 adherents. In 2012, a fruit and veg pattern and a meat and potato pattern were again identified with 64 and 73 people classified to these respectively. Although two similar patterns were identified at both time points, closer examination of the factor loadings revealed that the patterns changed. The “Fruit & Veg” pattern in 2012 no longer loaded on poultry, cruciferous vegetables or non-fried potatoes, and the loading for wine increased, while the negative loading for confectionery was no longer apparent. The “Meat & Potato” pattern was similar in both years but in 2012 the loadings for refined grains, takeaway foods and cakes, pastries and biscuits, and soya milk were below |0.2| and non-fried potato was above |0.2|. The third pattern in 2012 to which 46 women were classified was a “Veg & Soy Milk” pattern, characterised by a high intake yellow or red vegetables, cruciferous vegetables and soy milk. Figures visually display the changing factor loadings of dietary patterns between 1998 (Figure 27) and 2012 (Figure 28).

	1998			2012		
	1	**2**	**3**	**1**	**3**	**2**
Dietary Pattern	**Fruit & Veg**	**Meat & Potato**	**Meat & Dairy**	**Fruit & Veg**	**Meat & Potato**	**Veg & Soy**
	n = 63	n = 64	n = 56	n = 64	n = 73	n = 46
Eigenvalue	2.81708	1.45008	1.19767	2.21578	1.35168	1.70587
Variance	2.54134	1.78082	1.51674	2.11781	1.36579	1.85017
Proportion	0.465	0.3259	0.2775	0.4016	0.259	0.3509
Whole grains	-0.0259	-0.7450	0.1753	0.2007	**-0.3194**	0.0576
Refined grains	0.1717	**0.3431**	-0.1139	-0.0436	0.1843	-0.0414
Red meats	-0.0695	**0.3223**	0.2856	-0.0860	**0.4622**	0.0063
Processed meats	-0.1506	**0.4127**	0.0203	0.0771	**0.3674**	-0.1235
Poultry	**0.2099**	**0.3480**	**0.2741**	0.0309	**0.4024**	-0.0115
Takeaway foods	-0.0683	**0.2666**	-0.1129	-0.1303	0.1480	-0.1068
Fried fish	-0.0988	0.1549	0.1065	-0.0477	0.1425	-0.0806
Other fish	0.0115	-0.0548	0.0772	0.1643	0.1074	-0.0539
Fried potatoes	-0.1514	**0.2937**	0.0913	-0.1096	0.4713	-0.1083
Other potato	**0.2321**	0.0879	**0.2683**	-0.0325	**0.3425**	0.1556
Yellow or red vegetables	**0.6758**	0.0772	0.0474	-0.0678	0.0116	**0.8412**
Legumes	**0.4075**	0.1311	-0.1931	**0.2878**	0.1295	0.0708
Cruciferous vegetables	**0.4680**	0.0278	0.0998	0.1823	-0.0692	**0.6079**
Leafy green vegetables	**0.5140**	-0.1432	-0.1233	**0.6992**	-0.0096	-0.0453
Other vegetables	**0.6858**	0.0025	0.0629	**0.6594**	-0.0411	-0.0280
Tomato	**0.2966**	-0.1738	0.0211	**0.6045**	-0.1074	-0.0170
Fresh fruit	**0.4053**	**-0.3118**	0.0043	**0.4429**	**-0.2175**	-0.1268
Canned fruit	0.0529	-0.0085	0.0691	-0.1293	-0.0081	0.0422
Cakes, biscuits, sweet pastries	-0.1033	**0.2014**	**-0.5744**	-0.2521	-0.0909	-0.1338
Low-fat dairy products	0.0881	-0.0434	**0.3825**	0.0613	-0.0455	-0.0317
Full- fat dairy products	0.0870	0.0728	**-0.5025**	-0.0818	-0.1077	-0.1635
Soya milk	-0.1592	**-0.2257**	-0.0241	-0.1460	-0.0776	**0.6913**
Confectionery	**-0.2506**	0.0957	-0.0821	-0.0216	-0.1828	-0.1212
Added sugar	**-0.2464**	0.0084	**-0.2772**	-0.2383	-0.0296	-0.1709
Crisps	-0.0850	0.1218	0.0204	0.0320	**0.2767**	0.1084
Nuts	0.0726	-0.0691	0.0279	-0.0001	-0.1227	0.0235
Eggs	-0.0476	-0.0259	0.0690	0.1257	0.0133	0.0495
Fruit juice	-0.0654	-0.1791	0.0585	0.1430	-0.0536	-0.1157
Saturated spreads	0.0067	0.1339	**-0.2899**	-0.1667	-0.1468	0.1889
Unsaturated spreads	-0.1849	-0.1521	**0.3057**	0.0526	0.0036	-0.0483
Alcohol - beer	0.1510	0.1800	0.0037	0.0949	0.1107	0.0570
Alcohol - wine	0.1966	-0.0249	0.1664	**0.3802**	**0.2204**	-0.0222
Alcohol - spirits	-0.0738	-0.0049	0.1228	0.0238	0.1139	0.0940

Items in bold are food categories with a pattern-specific factor loading of > 0.2 or < -0.2

Table 30: Dietary pattern factor loadings for all WHAP participants in 1998 and 2012 (n = 183)

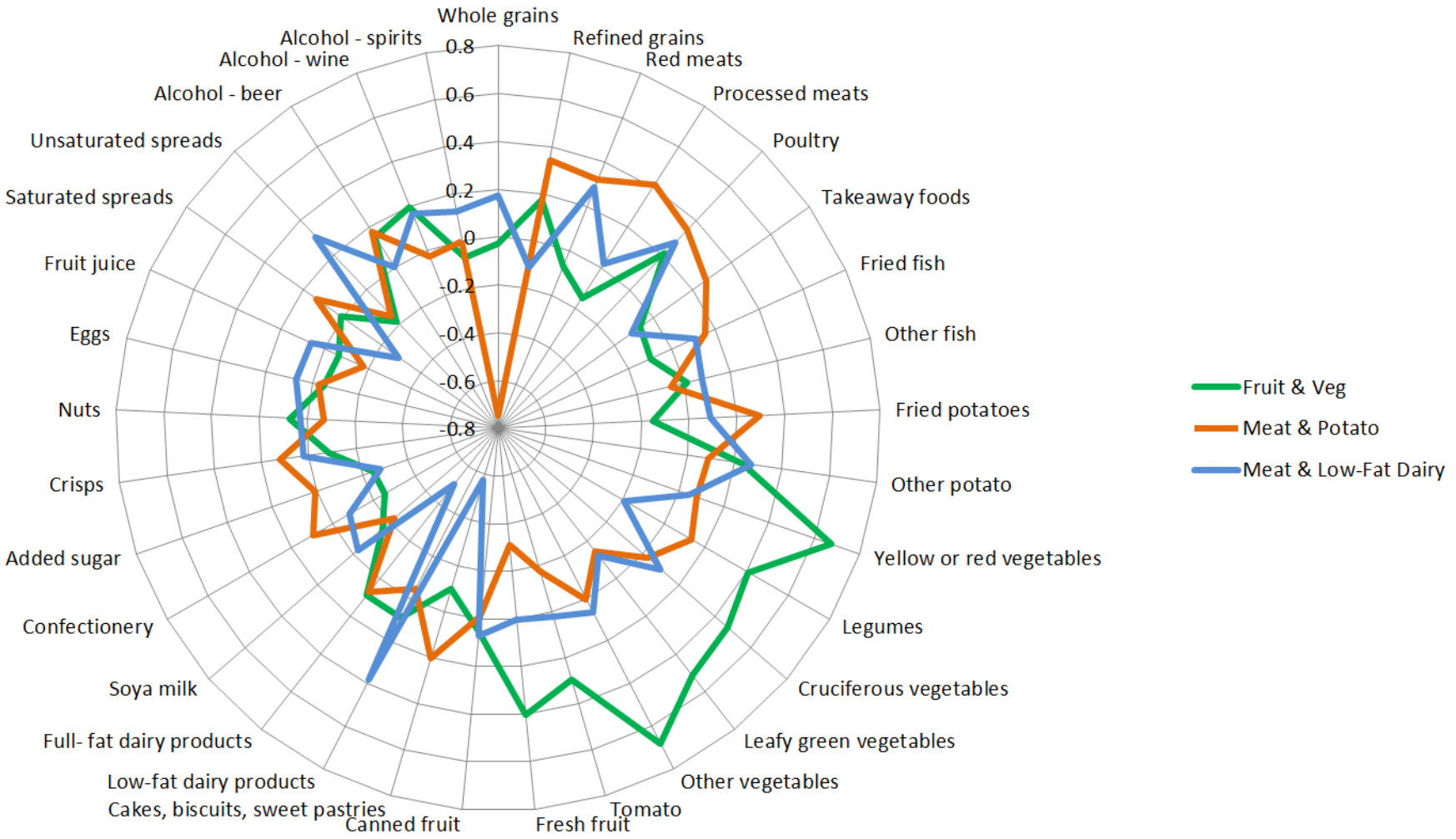

Figure 27: Spider diagram of factor loadings by dietary pattern derived by IPFA in 1998 (n = 183)

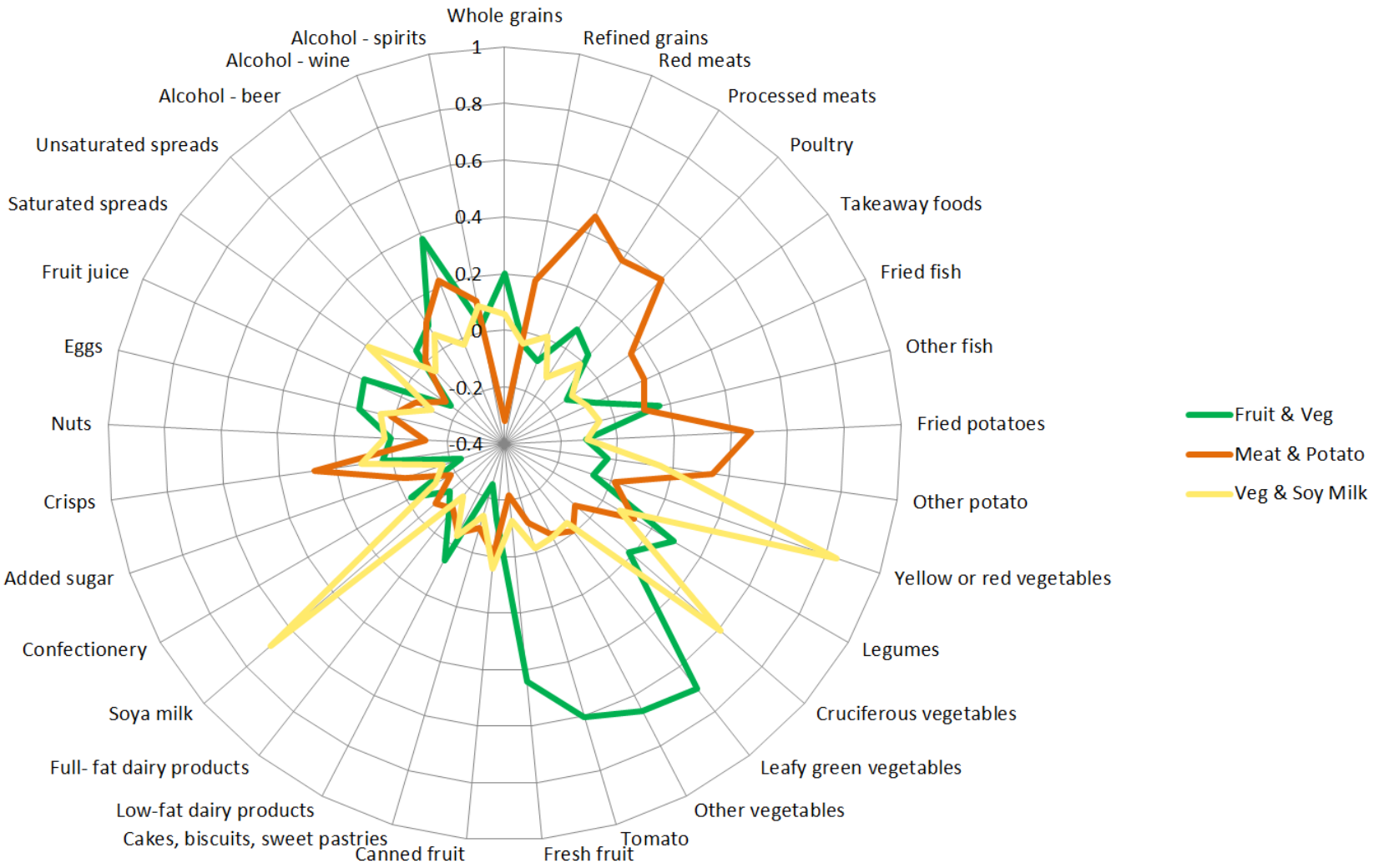

Figure 28: Spider diagram of factor loadings by dietary pattern derived by IPFA in 2012 (n = 183)

Demographic statistics stratified by dietary pattern adherence are displayed in Table 30. Participants adhering to a “Fruit & Veg” diet reported a higher level of education in 2012 (13.5 years) than “Meat & Potato” (11.9 years) or “Veg & Soy Milk” (12.4 years). In 1998, most of the “Fruit & Veg” (n = 31, 49.2%) adherers were within a healthy weight range (<= 25.0 kg/m2), while the majority of the “Meat & Potato” (n = 28, 43.8%) and “Meat & Low-Fat Dairy” (n = 23, 41.1%) adherers were overweight (>= 25.0 and < 30.0 kg/m2). In 2012, the majority of “Fruit & Veg” (n = 28, 43.8%) and “Veg & Soy Milk” (n = 19, 41.3%) adherers were overweight, whilst most of the “Meat & Potato” adherers (n = 27, 37.0%) were obese (>=30kg/m2). Change of smoking status was similar across all dietary patterns, with the percentage of non-smokers increasing for “Fruit & Veg” (84.1% in 1998 to 93.8% in 2012) and “Meat & Potato” (87.5% in 1998 to 89.0% in 2012).

		1998			2012		
Dietary Pattern		Fruit & Veg	Meat & Potato	Meat & Low-Fat Dairy	Fruit & Veg	Meat & Potato	Veg & Soy Milk
Participants		n = 63	n = 64	n = 56	n = 64	n = 73	n = 46
Age							
	Mean age in years	55.3 ± 2.3	55.6 ± 2.3	55.9 ± 2.4	69.2 ± 2.2	70.5 ± 2.5	69.8 ± 2.7
Marital Status							
	Single	2 (3.2%)	3 (4.7%)	1 (1.8%)	2 (3.1%)	3 (4.1%)	0 (0.0%)
	Married	45 (71.4%)	51 (79.7%)	46 (82.1%)	38 (59.4%)	50 (68.5%)	25 (54.4%)
	Divorced	9 (14.3%)	3 (4.7%)	6 (10.7%)	7 (10.9%)	5 (6.9%)	12 (26.1%)
	Separated	2 (3.2%)	3 (4.7%)	0 (0.0%)	1 (1.6%)	3 (4.1%)	2 (4.4%)
	Widowed	5 (7.9%)	1 (1.6%)	2 (3.6%)	14 (21.9%)	9 (12.3%)	4 (8.7%)
	Other	0 (0.0%)	3 (4.7%)	1 (1.8%)	2 (3.1%)	3 (4.1%)	3 (6.5%)
Education		Education reported in 2012					
	Education (including primary school) in years				13.5 ± 3.8	11.9 ± 3.3	12.4 ± 3.4
BMI category							
	Healthy weight (>= 18.5 and < 25kg/m^2)	31 (49.2%)	18 (28.1%)	21 (37.5%)	20 (31.3%)	21 (28.8%)	15 (32.6%)
	Overweight (>=25 and <30kg/m^2)	19 (30.2%)	28 (43.8%)	23 (41.1%)	28 (43.8%)	25 (34.3%)	19 (41.3%)
	Obese (>=30kg/m^2)	13 (20.6%)	18 (28.1%)	12 (21.4%)	16 (25.0%)	27 (37.0%)	12 (26.1%)
Smoking status							
	Current smoker	10 (15.9%)	8 (12.5%)	7 (12.5%)	4 (6.3%)	8 (11.0%)	2 (4.4%)
	Current non-smoker	53 (84.1%)	56 (87.5%)	49 (87.5%)	60 (93.8%)	65 (89.0%)	44 (95.7%)

BMI, body mass index. Expressed are numbers (%). Percentage is of time point population total. ± Indicates standard deviation

Table 31: Characteristics of WHAP study population in 1998 and 2012 (n = 183) by dietary pattern

Energy intake for the "Meat & Potato" pattern women in 1998 was more than 1000kJ higher than for the "Fruit & Veg" group, and higher than for the "Meat & Low-Fat Dairy" group (Table 32). In 2012, the "Meat & Potato" group still had a higher energy intake than the "Fruit & Veg" group but the highest reported intake was for the "Veg & Soy Milk" group. In 1998, women who were classified within the "Fruit & Veg" pattern consumed a higher proportion of fresh fruit, legumes, leafy green vegetables and other vegetables for mJ of energy intake than women in the other two groups. In 2012 the "Veg & Soy Milk" group ate relatively more yellow, red and cruciferous vegetables, at both time points whilst participants classified in the "Meat & Potato" pattern ate a higher proportion of red meats, processed meats, takeaway foods and fried potatoes relative to energy at both time points. The "Fruit & Veg" and "Meat & Potato" groups both tended to consume relatively more wine in 2012 than in 1998.

	1998			**2012**		
	Fruit & Veg	**Meat & Potato**	**Meat & Low-Fat Dairy**	**Fruit & Veg**	**Meat & Potato**	**Veg & Soy Milk**
	n = 63	**n = 64**	**n = 56**	**n = 64**	**n = 73**	**n = 46**
Energy (kJ/day)	5179.2 ± 217.5	6530.2 ± 358.6	5706.6 ± 211.5	4825.4 ± 203.4	5269.5 ± 218.3	5792.6 ± 193.8
Whole grains (g/MJ/day)	23.4 ± 1.5	10.1 ± 0.8	25.2 ± 1.3	21.2 ± 1.1	14 ± 1.2	23.7 ± 1.5
Refined grains (g/MJ/day)	14.2 ± 1.1	16.7 ± 1.3	10.9 ± 0.8	8.5 ± 0.6	11.1 ± 0.8	8.1 ± 1.4
Red meats (g/MJ/day)	5.6 ± 0.5	8.0 ± 0.5	7.4 ± 0.5	4.9 ± 0.4	8.7 ± 0.6	5.3 ± 0.5
Processed meats (g/MJ/day)	0.9 ± 0.1	2.2 ± 0.3	1.2 ± 0.1	1.2 ± 0.1	1.9 ± 0.2	0.8 ± 0.1
Poultry (g/MJ/day)	3.6 ± 0.3	3.3 ± 0.3	3.1 ± 0.2	2.5 ± 0.2	3.6 ± 0.3	2.4 ± 0.3
Takeaway foods (g/MJ/day)	3.5 ± 0.3	5.7 ± 0.5	3.5 ± 0.3	3.8 ± 0.4	5.4 ± 0.6	3.6 ± 0.7
Fried fish (g/MJ/day)	0.2 ± 0	0.6 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
Other fish (g/MJ/day)	4.1 ± 0.6	3.3 ± 0.4	3.8 ± 0.5	4.6 ± 0.4	3.9 ± 0.5	3.8 ± 0.4
Fried potatoes (g/MJ/day)	0.7 ± 0.1	2 ± 0.2	1.3 ± 0.2	1.0 ± 0.2	2.6 ± 0.3	1.0 ± 0.1
Other potato (g/MJ/day)	5.2 ± 0.5	4.5 ± 0.4	5.6 ± 0.7	4.1 ± 0.5	4.9 ± 0.6	5.0 ± 0.6
Yellow or red vegetables (g/MJ/day)	5.5 ± 0.3	2.9 ± 0.2	3.7 ± 0.3	4.3 ± 0.3	3.8 ± 0.3	6.5 ± 1.3
Legumes (g/MJ/day)	4.6 ± 0.4	3.7 ± 0.3	3.0 ± 0.2	5.0 ± 0.5	3.7 ± 0.3	4.3 ± 0.4

Cruciferous vegetables (g/MJ/day)	5.8 ± 0.5	3.0 ± 0.3	3.7 ± 0.3	4.8 ± 0.5	3.3 ± 0.3	7.1 ± 0.8
Leafy green vegetables (g/MJ/day)	3.1 ± 0.3	1.4 ± 0.1	1.9 ± 0.1	3.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.2
Other vegetables (g/MJ/day)	6.5 ± 0.4	3.0 ± 0.2	4.0 ± 0.2	6.3 ± 0.4	3.7 ± 0.2	4.1 ± 0.3
Tomato (g/MJ/day)	2.9 ± 0.3	1.4 ± 0.2	2.0 ± 0.2	4.3 ± 0.4	1.7 ± 0.2	2 ± 0.3
Fresh fruit (g/MJ/day)	53.1 ± 3	29.2 ± 1.9	37.8 ± 2.5	46.9 ± 2.4	27.8 ± 1.8	33.2 ± 2.9
Canned fruit (g/MJ/day)	2.0 ± 0.6	1.5 ± 0.4	1.9 ± 0.7	1.2 ± 0.3	1.7 ± 0.3	3.1 ± 0.8
Cakes, biscuits, sweet pastries (g/MJ/day)	2.6 ± 0.4	4.6 ± 0.5	1.8 ± 0.2	2.7 ± 0.3	3.7 ± 0.3	2.9 ± 0.4
Low-fat dairy products (g/MJ/day)	38.7 ± 4.9	34.3 ± 4.7	51.8 ± 4.3	45.5 ± 5	37.4 ± 4.1	58.7 ± 10.8
Full-fat dairy products (g/MJ/day)	23.2 ± 2.9	21.4 ± 2.7	10.4 ± 1.4	25.9 ± 2.8	27.6 ± 3.5	24.6 ± 3.6
Soya milk (g/MJ/day)	10.9 ± 2.9	7.4 ± 2.8	8.6 ± 5.7	5.5 ± 2.1	4.1 ± 1.6	15.2 ± 6
Confectionery (g/MJ/day)	0.4 ± 0.1	1.1 ± 0.2	0.6 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.1 ± 0.2
Added sugar (g/MJ/day)	1.4 ± 0.2	2.6 ± 0.3	1.6 ± 0.3	1.1 ± 0.2	2.0 ± 0.2	1.0 ± 0.2
Crisps (g/MJ/day)	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.1
Nuts (g/MJ/day)	0.5 ± 0.1	0.2 ± 0	0.4 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.2
Eggs (g/MJ/day)	2.2 ± 0.2	2.2 ± 0.2	1.9 ± 0.2	3.6 ± 0.3	3.1 ± 0.3	2.9 ± 0.3
Fruit juice (g/MJ/day)	9.9 ± 1.4	6.5 ± 1	10.5 ± 1.9	6.6 ± 1.3	4.4 ± 0.8	2.6 ± 0.7
Saturated spreads (g/MJ/day)	0.7 ± 0.2	1.0 ± 0.2	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	1.0 ± 0.2
Unsaturated spreads (g/MJ/day)	0.8 ± 0.2	0.9 ± 0.2	2.6 ± 0.3	1 ± 0.2	1.2 ± 0.2	1.0 ± 0.2
Alcohol beverage - beer (g/MJ/day)	4.3 ± 3	3.9 ± 2.1	2.8 ± 1.6	4 ± 2.9	1.9 ± 1.2	1.2 ± 0.4
Alcohol beverage - wine (g/MJ/day)	33.8 ± 5.3	17.1 ± 3.4	26.1 ± 4.1	46.5 ± 8.4	26.6 ± 3.8	20.2 ± 3.6
Alcohol beverage - spirits (g/MJ/day)	1.7 ± 0.4	2.4 ± 0.6	3.5 ± 0.9	1.4 ± 0.4	1.9 ± 0.8	0.9 ± 0.3

± Indicates standard deviation

Table 32: Energy adjusted mean DQES v2 daily intakes by dietary pattern identified by IPFA

Sixty-eight participants (37.2%) were classified in the same pattern at both time points. (Figure 29). Over 14 years of follow up, 31 (49.2%) participants remained stable in their adherence to the “Fruit & Veg” dietary pattern. Sixteen participants (25.4%) transitioned from loading highest on a “Fruit & Veg” to a “Meat & Potato” pattern, 16 (25.4%) also moved from a “Fruit & Veg” to a “Veg & Soy Milk” pattern. Of those who adhered to a “Meat & Potato” dietary pattern in 1998 (n = 64), the majority (n =

37, 57.8%) remained whilst 14 (21.9%) transitioned to a “Fruit & Veg” and 13 (20.3%) to a “Veg & Soy Milk” pattern. For the WHAP participants who were classified in the “Meat & Low-Fat Dairy” dietary pattern in 1998 (n = 56), 19 (33.9%) were classified in the “Fruit & Veg” pattern in 2012, 20 (35.7%) in the “Meat & Potato” and 17 (30.4%) in the “Veg & Soy Milk” pattern. Classifying the 1998 “Fruit & Veg”, 1998 “Meat & Low-Fat Dairy” and 2012 “Veg & Soy Milk” patterns as reasonably healthy, and the “Meat & Potato” pattern as considerably less healthy, these figures suggest that overall women tended to transition to a less healthy pattern over time.

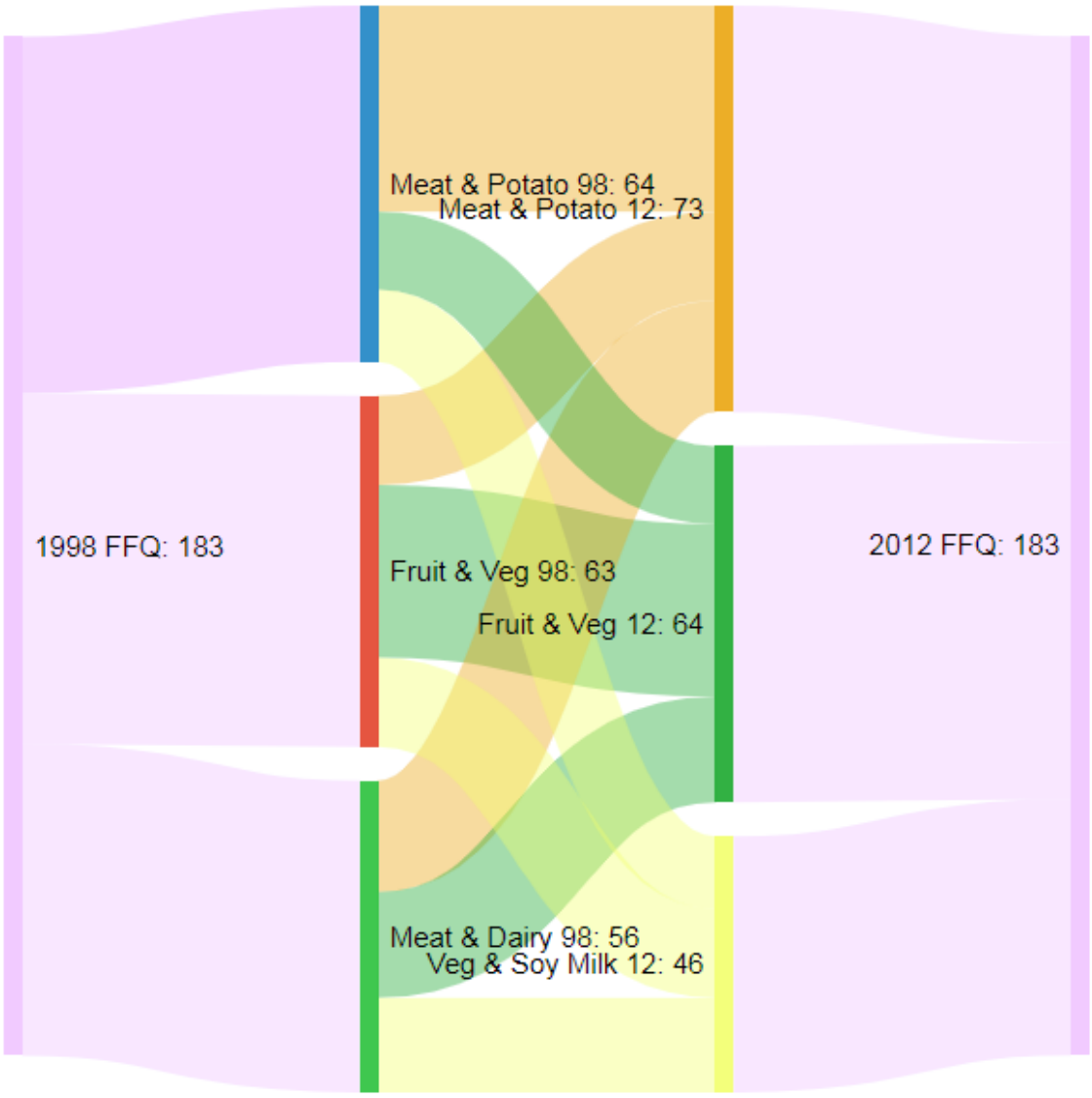

Figure 29: Sankey diagram for the stability of dietary patterns (Aussie, MeDi or Grazer) of WHAP participants (n = 183) between 1998 and 2012

5.4 Discussion

Using the same dietary instrument administered on two occasions approximately 14 years apart, two similar dietary patterns, one consistent with other 'healthy' patterns and one consistent with other 'unhealthy' patterns were identified. Although the time-specific loadings and amounts of different foods consumed varied over time, there was moderate stability of the dietary patterns over time. At both time points, a third dietary

pattern was identified but these were quite different at each time and loaded limited items. Although the number of women classified as adhering to each pattern did not change much over time, only 37% (68) of the 183 women remained classified in the same dietary pattern over time. Overall, there were more women in the "Meat & Potato" pattern in 1998 and 2012 which would be considered less healthy. This is consistent with our previous findings that indicated a trend towards a higher DII or lower Mediterranean diet score [285].These findings suggest women undergo dietary pattern changes over this stage of their lives.

An overall trend was observed in the direction of unhealthier dietary pattern classification. The majority of women were classified in the "Meat & Potato" pattern, a pattern consistent with other 'unhealthy' dietary patterns previously identified, such as the 'meat and chips' pattern identified by Marques-Vidal*, et al.* [350]. Although two patterns were consistently identified at both time points, the loadings were different over time. This indicates that even if an individual scored similarly on a pattern at both time points, it would not indicate they ate the same foods in the same proportions at both times. Furthermore, loadings do not always correspond with changes in intake. For example, the 1998 "Fruit & Veg" pattern the inverse loading for cakes, biscuits and sweet pastries decreased over time (-0.10 to -2.25); however, the intake of this food group increased for women classified in this pattern (2.6g/day to 2.7g.day). Relative to the increased intake of cakes, biscuits and sweet pastries seen in the other two food patterns, this is a small increase in the intake for women in the "Fruit & Veg" pattern.

In agreement with many other studies there was a trend for women classified in the healthiest pattern in 2012, to report more education than the women in the less healthy patterns. Consistent with the "Fruit & Veg" pattern representing the healthiest pattern, in 1998, 49.2% of women classified to this pattern were in a healthy weight range (>= 18.5 and < 25kg/m^2) compared with 28.1% in "Meat & Potato" and 37.5% in "Meat &

Low-Fat Dairy" patterns. The "Meat & Potato" pattern was associated with the highest proportion of obesity on both occasions but the difference in the proportion of healthy weight women across dietary patterns decreased over time, as did the proportion of healthy weight women. This increase in BMI is consistent with other studies [359] suggesting BMI changes may be more related to age than menopause, which most WHAP participants have experienced over the study period.

Alcohol consumption, predominantly wine, went from weak loadings in 1998 to loading on both the "Fruit & Veg" pattern and the "Meat & Potato" pattern, consistent with increased intake in both patterns. This is consistent with Australian national data from 1983, 1995 and 2011/12 surveys based on 24 hour dietary records showing that over time intake of alcohol decreased for most demographic groups but for women aged 45 years and over it increased [360]

Previous research has shown varying degrees of stability in dietary patterns over time, it may be the case that the age group analysed in this cohort are particularly susceptible to dietary changes as a result of sociodemographic change. Both Marques-Vidal*, et al.* [350] and Song*, et al.* [352] assessed longitudinal by analysing age-specific adherence to dietary pattern scores over time. Utilising adjusted and unadjusted dietary intakes, we were able to go one step further at looking at the individual food items that were responsible for driving the change observed in dietary pattern classification. This study was conducted on the same women using the same validated food frequency questionnaire in both 1998 and 2012, removing the possibility that reported changes are due to dietary methodology. As has been discussed previously, this study is unable to account for composition of food and beverages [328] and is limited by a small sample size.

In longitudinal dietary pattern analysis, many researchers assume dietary stability by measuring diet at one time point. This paper has shown that in ageing Australian women

dietary pattern classification undergoes substantial change over time and various lifestyle factors, other than simply dietary choices, may be driving that change. Sociodemographic, lifestyle and financial circumstances may have an impact on dietary pattern adherence, therefore suggesting long term diet-disease associations from cross-sectional dietary collection may be reaching incorrect conclusions.

6 DIETARY PATTERNS AND BETA-AMYLOID DEPOSITION

This chapter presents a cross-sectional analysis of the relationship between dietary pattern adherence and beta-amyloid deposition. Utilising data collected as part of the 2012 time point of the WHAP, this chapter presents the extrapolation of dietary pattern using factor analysis and the relationship between these patterns and cerebral Aβ deposition.

This chapter contains a modified version of the following publication[361]:

Hill E, Clifton P, Goodwill AM, Dennerstein L, Campbell S, Szoeke C. Alzheimer's Dementia. Dietary patterns and β-amyloid deposition in aging Australian women. 2018;4:535-41.

6.1 Introduction

Diet may play a substantial role in the Alzheimer's disease (AD) symptomatology and offer great potential for non-pharmacological prevention. Epidemiological evidence has suggested increased adherence to a Mediterranean diet [247], low glycemic index [239,248] and higher consumption of ω-3 polyunsaturated fatty acids [362] were associated with a decrease in AD biomarker burden. Evidence from systematic review found 50 out of 64 studies revealed an association between diet and AD incidence [236]; however, only one study has utilised *a priori* analysis to analyze dietary associations with the hallmark cerebral protein implicated in AD; beta amyloid (Aβ). In this study, dietary pattern analysis identified a pattern characterized by a higher intake of fresh fruit, vegetables, whole grains, fish and low-fat dairies and a lower intake of sweets, fried potatoes, processed meat and butter was negatively associated with *in vivo* cerebral Aβ [363].

Furthermore, male and mixed cohort studies predominate the research and, to date, no study has investigated this relationship specifically in women. Women are more likely than men to develop AD [364], have a higher penetrance for the apolipoprotein epsilon-4 allele (APOE-ε4) allele [365] and are more likely to progress from mild cognitive impairment (MCI) to AD [365]. Impacts of higher male mortality, vascular risk factors and the post-menopausal loss of estrogenic neuroprotection suggest females are 1.5 times more likely to develop AD than men [366]. Given sex differences in AD risk, research is needed for those at greater risk of disease.

Studies investigating *in vivo* AD biomarkers are needed in order to clarify how nutrition promotes healthy brain ageing and to identify neuroprotective patterns for those at the greatest risk of AD. The objectives of this study were to identify dietary patterns using an *a priori* approach and investigate their associations with Aβ deposition in healthy

ageing Australian women. We previously reported on a lack of a relationship between a healthy Mediterranean-diet and Aβ deposition [367] and hypothesized this was due to limitations of the self-reported food frequency questionnaire in measuring the potentially beneficial phytochemicals in olive oil. Given high-fat, high-glycemic diets have been associated with increased AD biomarker burden, we hypothesized that a dietary pattern characterized by high-fat, high-sugar content would be associated with an increase in cerebral Aβ pathology.

6.2 Methods

6.2.1 Study Population

Participants were sought from the 2012 follow-up of the Women's Health Ageing Project (WHAP), an epidemiologically sourced prospective study of healthy ageing Australian women. WHAP is an extension of the Melbourne Women's Mid-life Health Project (MWMHP). Briefly, 438 women within the Melbourne metropolitan area were identified by random digit dialling in 1991. Women were eligible for the cohort if they were Australian-born, aged 45-55 years, had menstruated in the three months prior to recruitment, and were not taking oestrogen-containing hormone replacement therapy. In 2012, participants were re-contacted and invited to participant in a late-life health study. Clinical assessments were conducted on 252 participants by trained field researchers. The clinical assessments included a battery of validated measures of physical health, sociodemographics, lifestyle, cognitive function, psychological health and biomarkers. A complete methodology has been published elsewhere [257].

6.2.2 Diet

Participants completed a validated food frequency questionnaire entitled the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES) [272]. The DQES v2 incorporates 80 food items with frequency response options on 74 of these items. The

DQES v2 covers five types of dietary intake; cereals/sweets/snacks, dairy/meat/fish, fruit, vegetables and alcoholic beverages. Data collected by the DQES v2 were used to calculate daily energy and nutrient intakes by the Cancer Council of Victoria (CCV) based on Australian nutrient composition data from NUTTAB95, collated via the Composition of Foods, Australia [273]. Individuals were removed if their energy intake was reported as below 3000 kJ/day or above 20,000kJ/day. All food items were reported in grams per day and placed into 33 food groups defined *a priori* (Table 33) that was similar to those used by others [326,327]. Dietary patterns were extrapolated from food groupings using iterated principal factor analysis (IPFA) with oblique varimax rotation due to the presumed inter-collinearity and non-independence of dietary patterns.

Food Group	**Items in the DQES v2**
Whole grains	All bran, bran flakes, high fibre white bread, muesli, multigrain bread, porridge, rye bread, Weet-Bix, wholemeal bread
Refined grains	Corn flakes, crackers, pasta, rice, white bread
Red meats	Beef, lamb, pork, veal
Processed meats	Bacon, salami, sausages
Poultry	Chicken
Takeaway foods	Hamburger, meat pies, pizza
Fried fish	Fried fish
Other fish	Fish (non-fried), tinned fish
Fried potatoes	Chips (French fries)
Other potato	Potatoes
Yellow or red vegetables	Capsicum, carrots, pumpkin
Legumes	Baked beans, green beans, other beans, peas, tofu
Cruciferous vegetables	Broccoli, cabbage, cauliflower
Leafy green vegetables	Lettuce, spinach
Other vegetables	Bean sprouts, beetroot, celery, cucumber, garlic, mushrooms, onion, zucchini
Tomato	Tomatoes

Fresh fruit	Apples, apricots, avocado, bananas, mango, melon, oranges, peaches, pears, pineapple, strawberries
Canned fruit	Tinned fruit
Cakes, biscuits, sweet pastries	Cakes, sweet biscuits
Low-fat dairy products	Flavoured milk drink, low-fat cheese, reduced fat milk, ricotta cheese, cottage cheese, skim milk
Full- fat dairy products	Cream cheese, firm cheese, full-cream milk, hard cheese, ice cream, soft cheese, yoghurt
Soya milk	Soya milk
Confectionery	Chocolate
Added sugar	Jam, sugar
Crisps	Crisps
Nuts	Nuts, peanut butter
Eggs	Eggs
Fruit juice	Fruit juice
Saturated spreads	Butter, butter-margarine blends, margarine
Unsaturated spreads	Monounsaturated margarine, polyunsaturated margarine
Alcohol - beer	Heavy beer, light beer
Alcohol - wine	Red wine, white wine
Alcohol - spirits	Fortified wines, spirits

Table 33: Food groupings from Dietary Questionnaire for Epidemiological Studies Version 2

6.2.3 Imaging

In the 2012 follow-up all WHAP participants were offered the opportunity to have cerebral imaging. Aβ deposition was measured via *in vivo* F-18 Florbetaben positron emission tomography (PET) at the Austin Health Centre for PET in Victoria, Australia. Participants received 250 MBq of 18F-FBB intravenously, with a 20-minute acquisition commencing 90-minutes post injection. Standardised uptake values (SUV) were calculated for all brain regions examined and standard uptake value ratios (SUVR) were generated by normalising regional SUV by the cerebellar cortex with atrophy-correction

from structural magnetic resonance imaging (MRI). Neocortical SUVR, a global index of Aβ burden, is expressed as the average SUVR of the area-weighted mean. Area weighted means were calculated for each participant by averaging the frontal, superior parietal, lateral temporal, lateral occipital and anterior and posterior cingulate regions. This protocol has been described elsewhere [257].

6.2.4 Covariates

Age (in years), education (in years) and body mass index (BMI) were collected as part of the clinical assessments in 2012. Total energy intake in kilojoules was calculated by the CCV from the DQES v2. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Savings Score was utilized as a valid indicator of cognitive ability [368], it has been suggested as the most reliable index in differentiating cognitively normal individuals from AD [369]. Participants' APOE genotype was determined by direct sequencing and were dichotomizewered as an APOE ε4 carrier (APOE ε2/ε4, APOE ε3/ε4 and APOE ε4/ε4) or a non-carrier. Adherence to identified dietary patterns was converted from weighted factor loadings to binary adherence to minimize the intra-correlations between variables. All analyses were adjusted for age in years, education in years, energy intake (kJ/day), cognition (CERAD Savings) and binary presence of the APOE ε4 allele.

6.2.5 Statistical Analysis

All analyses were conducted in STATA software on Windows operating system. Complete data were available for 115 WHAP participants and there were no significant differences between the included (n = 115) and excluded (n = 137) cohorts. PET SUVR displayed a positive skew that was rectified using 1/square transformation therefore results should be interpreted as inverse coefficient derivatives. Generalized linear models (GLM) were used to assess associations between Aβ deposition and dietary

patterns scores. GLMs were adjusted for age in years, education in years, cognition (CERAD Savings) and binary presence of the APOE ε4 allele.

6.3 Results

Four dietary patterns were identified: High-Fat, Mediterranean, Junk Food and Low-Fat. Factor loadings (Table 34 and Figure 30) indicated the High-Fat diet loaded heavily on food groups such as processed meats, fried fish, red meats, fried potatoes and poultry. The Mediterranean style diet loaded chiefly on whole grains, vegetables, nuts, fish and wine as the main source of alcohol. The unhealthy Junk-Food pattern was characterized by high consumption of takeaway foods, added sugar, confectionary and cakes, biscuits and sweet pastries, whilst the Low-Fat diet loaded heavily on low-fat dairy products, vegetables and unsaturated spreads.

Variable	**High-Fat**	**Mediterranean**	**Junk-Food**	**Low-Fat**
Eigenvalue	3.03802	2.13623	1.80738	1.55595
Variance	2.15252	2.00648	1.58108	1.39511
Proportion	0.3164	0.2949	0.2324	0.2051
Whole grains		0.3668	0.2096	0.1387
Refined grains	0.1598	0.1673	0.1247	
Red meats	0.5464			0.1128
Processed meats	0.7281			
Poultry	0.4130	0.1302		0.1549
Takeaway foods	0.2021	-0.2543	0.3427	
Fried fish	0.4662	0.2473		
Other fish	0.4029	0.3867		
Fried potatoes	0.4195			
Other potato	0.1803		0.1127	0.3143
Yellow or red vegetables	0.1675	0.2594	0.1480	0.5034
Legumes			0.2923	0.3134
Cruciferous vegetables		0.2117	-0.1027	0.4114
Leafy green vegetables		0.5466		
Other vegetables	0.1961	0.6869		0.1286
Tomato	-0.2459	0.2255		
Fresh fruit	-0.2786	0.3381	0.1887	
Canned fruit			0.1337	

Cakes, biscuits, sweet pastries			0.6350	
Low-fat dairy products	-0.2222			0.5117
Full- fat dairy products	0.1754		0.1255	-0.4775
Soya milk		0.1843		-0.1101
Confectionery	-0.1195	0.2484	0.5211	
Added sugar	0.1263		0.4327	
Crisps	0.1405	0.1229		
Nuts		0.4485	0.1703	
Eggs	0.1083	0.1448		
Fruit juice	-0.1134			
Saturated spreads				-0.2113
Unsaturated spreads				0.2165
Alcohol - beer	0.1540	-0.1618		-0.1272
Alcohol - wine	0.1345	0.1586	-0.2868	

*Rotated factor loadings for iterated principal factor analysis with oblique promax rotation. Blanks represent absent loadings (<0.1). Alcohol – spirits not shown due to not loading (>0.1) on any factor.

Table 34: Dietary pattern factor loadings for all WHAP participants in 2012

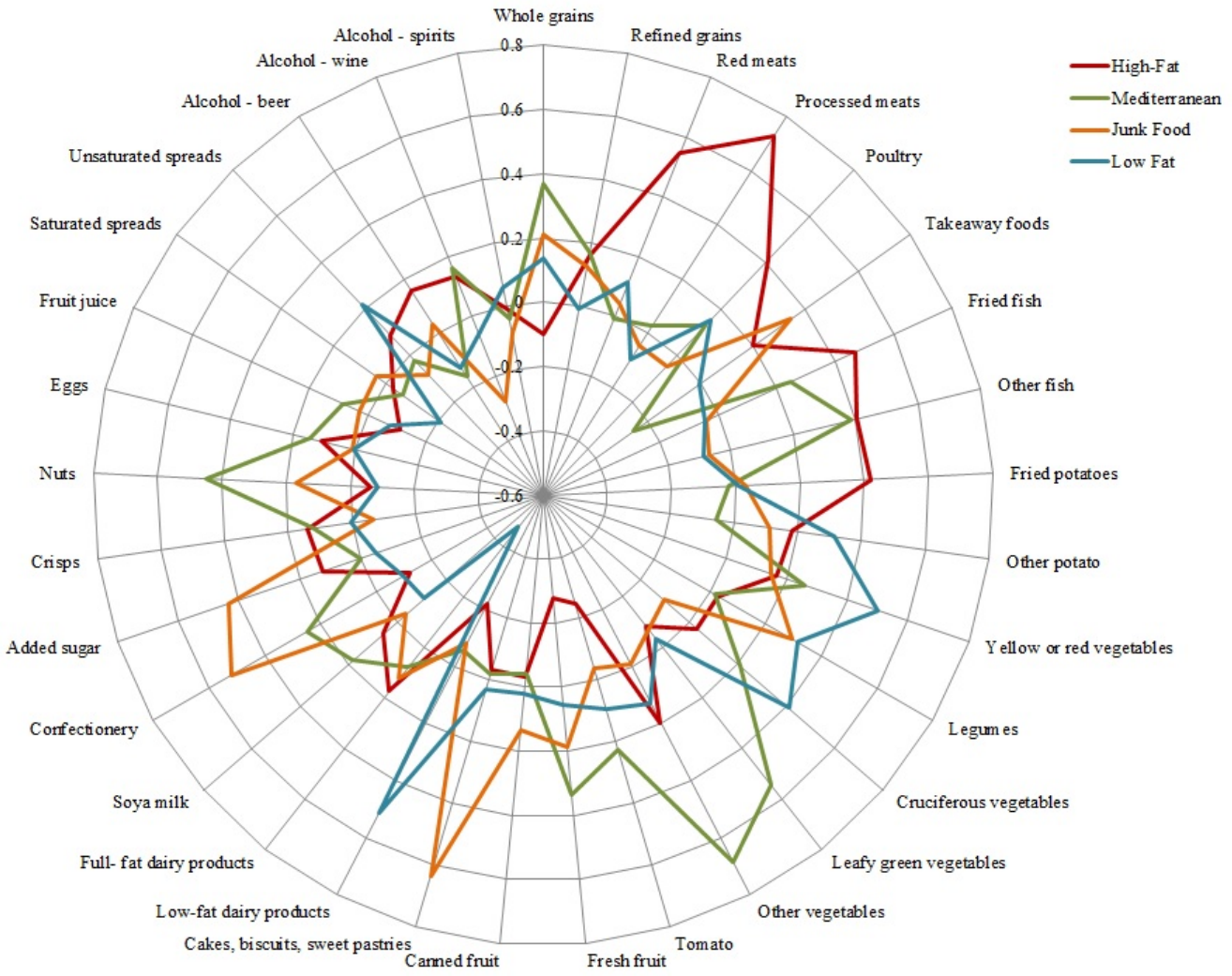

Figure 30: Spider diagram of factor loadings by dietary pattern

Participants' characteristics are found in Table 35. Participants in the Mediterranean diet group (n = 31) displayed the highest level of education (14.10 ± 3.87 years), highest CERAD Savings score (72.97 ± 31.07) and lowest level of Aβ deposition (PET SUVR 1.0834 ± 0.14). Daily energy intake was highest in the High-Fat group (5443.46 ± 2116.50 kJ/day) and lowest in the Mediterranean group (4677.26 ± 1242.79 kJ/day). Significant group differences were observed in education, energy intake and CERAD Savings and were therefore adjusted for in all GLMs.

Adherence to the Junk-Food diet was a significant predictor of Aβ deposition (β = .-10, p=0.036) as was binary presence of the APOE ε4 allele (β = -.11, p=0.004) (Table 36). No significant interaction effects were observed in the combined effect of diet and APOE ε4 on Aβ deposition (p=0.59). All other dietary patterns were not associated with Aβ deposition. Age, education and cognition were also not significantly associated with Aβ deposition.

	High-Fat (n = 24)	**Mediterranean (n = 31)**	**Junk Food (n = 24)**	**Low Fat (n = 35)**	**Total (n = 115)**	**Difference**
Age (in years)	69.79 ± 2.42	69.45 ± 2.23	70.41 ± 3.19	69.57 ± 2.70	69.76 ± 2.63	p=0.68
Education (in years)	12.88 ± 3.67	14.10 ± 3.87	11.50 ± 2.96	12.63 ± 3.36	12.84 ± 3.57	**p=0.03**
BMI	28.58 ± 6.75	27.43 ± 5.48	27.14 ± 5.58	29.29 ± 4.26	28.18 ± 5.46	p=0.39
Energy(kJ/day)	5443.46 ± 2116.50	4809.79 ± 1145.51	6035.40 ± 1993.21	4677.26 ± 1242.79	5160.53 ± 1679.03	**p=0.01**
APOE Positive n (%)	9 (37.5%)	9 (29.0%)	9 (37.5%)	10 (28.57%)	37 (32.46%)	p=0.73
Healthy Cognition* n (%)	24 (100%)	31 (100%)	22 (92%)	33 (94%)	110 (96%)	p=0.20
CERAD Savings Score %	72.93 ± 18.08	72.97 ± 31.07	65.57 ± 28.76	63.34 ± 28.62	68.45 ± 27.52	p=0.06
PET SUVR (Raw)	1.1296 ± 0.1539	1.0835 ± 0.1427	1.2150 ± 0.2458	1.1300 ± 0.2336	1.1352 ± 0.2026	p=0.10
PET SUVR (Transformed)	0.8185 ± 0.1770	0.8829 ± 0.1646	0.7389 ± 0.2174	0.8446 ± 0.2043	0.8273 ± 0.1959	**p=0.03**

If not otherwise described, data are presented as mean ± standard deviation of the mean. Comparison for categorical variables with chi-squared, continuous variables with one-way ANOVA. PET SUVR (Raw) was nonparametrically tested using Kruskal-Wallis test. * Healthy cognition refers to a non-MCI, non-AD memory complainer and or a non-MCI, non-AD non-memory complainer. Bold indicates statistical significance ($p<0.05$).

Table 35: Descriptive statistics for the included participants grouped by adherence to dietary patterns identified using IPFA

PET SUVR					
	Coefficient	**Std. Err.**	**p**	**C.I Lower**	**C.I Higher**
High-Fat	-0.00705	0.04372	0.872	-0.09273	0.07864
Mediterranean	0.06390	0.04349	0.142	-0.02135	0.14915
Junk Food	-0.09740	0.04511	**0.031**	-0.18582	-0.00898
Low-Fat	0.02338	0.03962	0.555	-0.05428	0.10103
Age (in years)	-0.00120	0.00702	0.864	-0.01495	0.01256
Education (in years)	0.00139	0.00502	0.781	-0.00845	0.01125
BMI	-0.00076	0.00326	0.816	-0.00714	0.00563
Energy (kJ/day)	-0.00001	0.00001	0.309	-0.00003	0.00001
APOE Presence	-0.10916	0.03919	**0.005**	-0.18598	-0.03233
CERAD Savings Score	0.00125	0.00065	0.054	-0.00002	0.00252

* Bold indicates statistical significance ($p<0.05$). Confidence intervals (95%) (C.I) are for coefficient. Analysis adjusted for age in years, education in years, cognition (Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Savings score) and binary presence of the APOE ε4 allele.

Table 36: Generalised Linear Model for independent variable (PET SUVR) and four dietary patterns identified using iterative principal factor analysis.

6.4 Discussion

In this cross-sectional study in Australian women, adherence to the Junk Food was a significant predictor of cerebral Aβ deposition. These results suggest that higher adherence to a high-fat, high-sugar style diet may be associated with an increased deposition of AD biomarkers and a higher risk for disease.

Although age, education and cognition were not significant predictors of cerebral Aβ deposition, we observed similar cognitive status between dietary groups. However, women adhering to the Mediterranean dietary pattern displayed significantly higher cognitive scores than the other dietary groups. In the epidemiological, longitudinal, cohort Nurse's Health Study, women with higher Mediterranean diet adherence had significantly higher overall cognitive status [370]. Given evidence for the cardiovascular determinants of cognitive decline [371,372] there is evidence for an inverse relationship between Mediterranean diet adherence and cognition; however, the cross-sectional nature of this study limits our ability to address this relationship.

Our results contribute to the growing body of evidence linking diet with AD. A high-glycemic diet has been associated with greater amyloid burden in brain [248] and cerebrospinal fluid (CSF) measures [239,241,373]. A principal component analysis on nutrient intake patterns showed consumption of omega-3 fatty acids, zinc, vitamin B-12 and vitamin D were associated with decreased amyloid deposition [209,246]. Consumption of omega-3 fatty acid supplementation has been shown to be related to tau (phosphorylated & total) and amyloid biomarkers of AD in CSF [374]. Serum docosahexaenoic acid has also been inversely associated with cerebral amyloid burden [375].

Research has established that diets with higher consumption of sugar, carbohydrates and high-glycemic foods are associated with impaired glucose metabolism [376]. Disrupted

glucose metabolism affects the production and clearance of Aβ and tau phosphorylation [377] and both insulin resistance [378] and type-2 diabetes [379] are risk factors for AD. Several animal studies have illustrated that a high-fat diet causes brain Aβ accumulation in wild type rabbits [380] and transgenic mice [381,382]. Furthermore, human APOE isoforms have been shown to modulate glucose and metabolic pathways, with the APOE ε3/ ε4 variants showing markedly reduced glucose uptake and metabolism in mouse models [383]. APOE ε2 brains demonstrated a more robust metabolic profile than APOE ε3/ε4, suggesting a physiological mechanism for its protective role against AD [383].

We speculate that the relationship observed between a high-fat, high-sugar diet and increased cerebral Aβ deposition may be modulated by impaired glucose metabolism in this female only cohort. We believe our results suggest an impaired glucose metabolic pathway interacting with an APOE-Aβ physiological mechanism. Research has shown impaired glucose metabolism is related to cerebral Aβ [384] and that APOE ε4 confers a greater risk in women than men [365]. Women with a single APOE ε4 allele have up to a 4-fold increase in risk when compared to women homozygous for APOE ε3; however, men with a single APOE ε4 allele have little to no increase in risk [385]. Given animal model evidence for an APOE mediated glucose metabolism [383], females may experience greater AD risk due to a mechanistic impairment in their glucose metabolism. Further research is required to elucidate the physiological mechanisms that underpin this relationship, for example to replicate animal evidence of glucose metabolism in human models of APOE ε4 isoforms.

Our findings strengthen the hypothesis of diet being a modifiable risk factor for AD by linking amyloid deposition with an unhealthy type diet in a female only cohort. These findings suggest a metabolic pathway linking diet with cerebral Aβ deposition and should

motivate investigations into dietary impacts on glucose metabolism by variations in presence of the APOE ε2/ε3/ε4 alleles.

7 Discussion & Conclusions

This final chapter presents a comprehensive discussion of the research undertaken within this thesis. A summary of the results will be presented, including comparisons and contrasts with existing literature in the field. Strengths and limitations of the work will be described, as well as an ultimate conclusion to be drawn. Finally, future directions will be presented.

7.1 Introduction

AD is the most common form of dementia and, with 40-50 million people currently living with dementia [1,2], is on track to reach global epidemic status over the next 30 years. Projections indicate, for the first time in history, the population of elderly people (aged 65 years or older) will double that of children (aged 0-14 years) before the year 2050[4]. Amidst our aging population, there will be an estimated 115.4 million people with AD by 2050[1]. Global deaths due to dementia rose by 148% between 1990 and 2016[5]. Currently, dementia is the fifth leading cause of death in the world[5] and the leading cause of death in women in Australia[6].

AD is a complex neurological disorder characterised by the two hallmark pathological markers; the extracellular deposition Aβ protein and the intracellular phosphorylation of tau into NFTs. This abnormal pathology is believed to contribute to the synaptic dysfunction and neuronal death that ultimately results in cognitive impairment. However; there is a preclinical window between neuropathological and clinical manifestations of AD that provides an opportunity to investigate potentially disease modifying therapies. Lacking a pharmacological treatment with proven efficacy, the drive to examine lifestyle risk factors that may alter AD progression is clear. Identifying lifestyle factors that may delay or prevent the onset of AD is a research focus that displays enormous therapeutic potential. As an accessible, cost-effective and non-pharmacological modifiable risk factor, an individual's diet may contribute to AD risk and therefore is a worthwhile research endeavour.

There is a growing body of evidence suggesting diet is an additional risk factor for AD. Evidence amassed from systematic review[141] suggests an association between diet and AD that may offer therapeutic potential. Given individuals consume diets that contain nutrients and non-nutrient foods, dietary pattern analysis may offer methodological

advantages over analysing individual food-disease relationships [387]. In AD nutritional epidemiology, *a posteriori* methods of dietary pattern analysis offer exploratory methods to identify patterns within a population, allowing interpretation of the synergistic effects of food combinations.

The present thesis aimed to further the growing body of knowledge surrounding the relationship between diet and AD. To achieve this aim, the initial objective was to collate and interpret the available evidence. A systematic review and meta-analysis was conducted to examine the association between diet and biomarkers of AD. Longitudinal dietary patterns and changing nutritional intakes were analysed over 14 years of follow up in WHAP participants, an epidemiologically sampled healthy cohort of women in the Melbourne metropolitan area. Finally, the relationship between dietary patterns and beta-amyloid was investigated in the WHAP cohort.

7.2 Summary of results

The main summary of results from this thesis is as follows:

- From the literature review conducted in this thesis, the overwhelming majority of studies investigating diet and AD have found a significant relationship. Studies consistently point to a neuroprotective effect of dietary patterns, for example studies that investigate the MeDi's protective effect overwhelmingly find decreased odds of AD development and mortality.
- From the systematic review and meta-analysis conducted in this thesis, the overwhelming majority of studies investigating diet and biomarkers of AD (tau and beta-amyloid) found a significant relationship. As part of the systematic review, 4 studies found a high-glycemic load was associated with an increase in AD biomarker burden and 6 found adherence to a MeDi or 'AD-protective' dietary pattern conferred

a reduction in AD biomarker burden. Meta-analysis revealed a small, yet significant, effect of diet on AD biomarkers. This systematic review provided support to the clinical evidence for a diet-AD relationship, by providing evidence for this relationship extending to the abnormal neuropathological development phase that precedes clinical manifestations by up to decades.

- The initial results chapter of this thesis presented an investigation into prospective changes in nutritional habits in WHAP participants over 14 years of follow up. Differences in nutritional intakes, DII, MeDi adherence, sociodemographic and physical measures were analysed in 1998 and 2012. Energy intake significantly decreased over time, whilst energy-adjusted total fat, saturated fat, monounsaturated fat and cholesterol intakes all significantly increased. MeDi adherence scores significantly decreased whilst DII scores non-significantly increased slightly, indicating changes in diet were consistently in the direction of a poorer diet.
- The second results chapter of this thesis provided a longitudinal dietary pattern analysis to assess dietary change over 14 years of follow up. Participants of the WHAP completed the same dietary questionnaire (DQES v2) in 1998 and 2012 and dietary patterns were extrapolated using IPFA. In 183 WHAP participants, three dietary patterns were identified at both time points; two ‘healthy-type’ patterns as well as a third less healthy pattern. In these women, although some participant’s dietary pattern remained largely stable over time, the majority of women underwent dietary pattern changes over this time in their lives.
- The third and final results chapter of this thesis presented a cross-sectional analysis of the relationship between dietary pattern adherence and beta-amyloid deposition. Utilising available dietary and neuropathological data collected as part of the 2012 time point of the WHAP, this chapter presented the extrapolation of four dietary

patterns using factor analysis; high fat, Mediterranean, junk food, and low fat. Adherence to the junk food diet was found to be a significant predictor of Aβ deposition in the WHAP cohort. This chapter has been cited by systematic review[388] and original research[389].

7.3 Changing diet

Chapters 4 & 5 explored the changing dietary habits of Australian women over 14 years of follow up. Between 1998 and 2012, nutritional and dietary patterns were consistently in the direction of a poorer diet. Energy intake significantly decreased over time and several absolute and energy-adjusted nutrients and macronutrients showed significant change. Scores on both the DII and MeDi displayed changes toward a poorer diet quality between the 1998 and 2012 time points. DII values tended to increase non-significantly, becoming more pro-inflammatory over time. Dietary pattern analysis identified two similar dietary patterns, one consistent with other 'healthy' patterns and one consistent with other 'unhealthy' patterns. Although the time point specific loadings and amounts of foods consumed varied between 1998 and 2012, there was moderate stability of the dietary patterns over time. The number of women classified as adhering to each pattern did not change substantially over 14 years of follow up, only 36% (n = 68) of the 183 remained classified in the same dietary pattern between time points. These findings indicate that women undergo substantial dietary pattern changes over this stage of their lives, and that this change is consistently in the direction of a poorer diet.

Energy intake decreased significantly over time. This is similar to the findings of a British study in adults over 17 years of follow up that found a significant decrease in energy consumption for both men and women between the ages of 43 and 53 years [328]. However this study, led by Prynne*, et al.* [328], also described an increase in energy intake between the ages of 36 and 43 years. They also found fat intake, as a percentage of energy,

decreased over time; however, the current study found significant increases in the contribution of fat to energy intake in Australian women from an average age of 55 to 70 years. In the current study there was a non-significant trend for vitamin C intake to decrease and vitamin E intake to increase, however, Prynne*, et al.* [328] found energy-adjusted increases for both reflecting an overall change to a diet more closely reflecting dietary guidelines. Carbohydrate intake significantly decreased over time in the WHAP participants; in contrast to the findings of Prynne*, et al.* [328] where women's total carbohydrate and percentage of energy from carbohydrate intake gradually increased over a period of 17 years (aged 36 to 53 years). Given the overall trend in the birth cohort studied by Prynne*, et al.* [328] towards a more healthy diet over time, it is possible the increase in carbohydrate intake was in favour of more complex carbohydrates, although this was not noted in their results.

Nutritional changes over 14 years of follow up in the WHAP cohort towards a less healthy diet were reflected in the BMI shifting towards obesity in the context of declining physical activity and reduced energy requirements with age [330]. Widowhood was also found to become more prevalent over 14 years, with participants who were widowed more likely to decrease MeDi adherence compared with participants experiencing a stable marital situation. Widowhood can have a dramatic impact on the surviving spouse, catalysing changes in daily routines and dietary choices that may adversely affect their nutritional status [298]. Widowed individuals are more likely to consume non-nutritious foods, fewer vegetables and are less likely to prepare homemade meals than their married peers [298,299,331,332]. The loss of a spouse also is related to a decrease in dietary diversity [331,332], reflecting a deprioritization of personal nutrition.

Compared to Prynne*, et al.* [328], who reported a trend towards a better diet over time, the current study observed a trend towards poorer diet quality. In the WHAP cohort,

consumption of fresh fruit and refined grains significantly decreased over time and several vegetable groups displayed non-significant decreases over 14 years. Given similar changes were observed for Anglo-Celt and Greek-Australian men and women aged above 70 years[323], the differences between WHAP and the UK cohort studied by Prynne*, et al.* [328] may be age-related. Aged between 36 and 53 years, the UK cohort[328] was at lower risk for the loss of employment, physical function, widowhood and social engagement than would be expected of women aged between 55 and 69 years. Compared to other studies such as Shivappa*, et al.* [276], scores and range of the DII values were low.

Consistent with nutritional, DII and MeDi adherence change over 14 years, an overall trend was observed in the direction of unhealthier dietary pattern classification. Two 'healthy-type' patterns (identified as "Fruit & Veg" and "Meat & Potato") were observed on both occasions. A third, less healthy pattern, was observed in 1998 and 2012, but these patterns were not similar. Although two patterns were consistently identified at both time points, the loadings were different over time. This indicates that even if an individual scored similarly on a pattern at both time points, it would not indicate they ate the same foods in the same proportions at both times.

Overall, there were more women in the "Meat & Potato" pattern in both 1998 and 2012 which would be considered less healthy. This is consistent with previous 'unhealthy' dietary patterns identified, such as the "meat and chips" pattern identified by Marques-Vidal*, et al.* [350]. In agreement with previous research, there was a trend for women classified in the healthiest pattern in 2012 to report more education than the women in the less healthy patterns. Consistent with the "Fruit & Veg" pattern representing the healthiest pattern, in 1998, 49.2% of women classified to this pattern were in a healthy weight range (>= 18.5 and < 25kg/m^2) compared with 28.1% in "Meat & Potato" and 37.5% in "Meat & Low-Fat Dairy" patterns. The "Meat & Potato" pattern was associated

with the highest proportion of obesity on both occasions but the difference in the proportion of healthy weight women across dietary patterns decreased over time, as did the proportion of healthy weight women. This increase in BMI is consistent with other studies[359] suggesting BMI changes may be more related to age than menopause, which most WHAP participants have experienced over the study period.

Alcohol consumption, predominantly wine, went from weak loadings in 1998 to loading on both the "Fruit & Veg" pattern and the "Meat & Potato" pattern, consistent with increased intake in both patterns. This is consistent with Australian national data from 1983, 1995 and 2011/12 surveys based on 24 hour dietary records showing that over time intake of alcohol decreased for most demographic groups but for women aged 45 years and over it increased [360].

Through dietary and nutritional analysis, WHAP participants appear to be transitioning towards an unhealthy diet over time, from approximately age 55 to 70 years. Although some of the women's dietary pattern adherence remained relatively stable, the majority of women underwent substantial dietary pattern changes over this stage of their lives. Adherence to the 'healthy-type' MeDi significantly decreased, whilst scores on the DII increased over 14 years of follow up between 1998 and 2012.

7.4 Diet and beta-amyloid

Chapter 6 explored the cross-sectional association between dietary pattern adherence and accumulation of the hallmark AD protein, Aβ. Factor analysis on those who had available dietary, neuropathological and covariate data identified four dietary patterns: High-Fat, Mediterranean, Junk Food and Low-Fat. Adherence to the Junk Food was found to be a significant predictor of cerebral Aβ deposition.

Cognitive status was similar between dietary pattern groups. However, women adhering to the MeDi pattern displayed significantly higher cognitive scores than the other dietary groups. In the longitudinal Nurse's Health Study, women with higher MeDi adherence had significantly higher overall cognitive status [370]. Given evidence for the cardiovascular determinants of cognitive decline [371,372] there appears to be clear evidence for an inverse relationship between MeDi adherence and cognition; however, this line of inquiry was outside the scope of this thesis.

This thesis chapter presented research that indicated adherence to a Junk Food diet was a significant predictor of Aβ accumulation, contributing to a growing body of evidence linking diet to biomarkers of AD. Previous research has suggested a high-glycemic diet is associated with greater amyloid burden in brain [248] and cerebrospinal fluid (CSF) measures [239,241,373]. A principal component analysis on nutrient intake patterns showed consumption of omega-3 fatty acids, zinc, vitamin B-12 and vitamin D were associated with decreased amyloid deposition [209,246]. Consumption of omega-3 fatty acid supplementation has been shown to be related to tau (phosphorylated & total) and amyloid biomarkers of AD in CSF [374]. Serum docosahexaenoic acid has also been inversely associated with cerebral amyloid burden [375].

Previous research has suggested diets with a higher consumption of sugar, carbohydrates and high-glycemic foods are associated with impaired glucose metabolism [376]. In previous studies, disrupted glucose metabolism has been shown to affect the production and clearance of Aβ and tau phosphorylation [377] and both insulin resistance [378] and type-2 diabetes [379] are risk factors for AD. Animal models have illustrated that a high-fat diet is causal for brain Aβ accumulation in wild type rabbits [380] and transgenic mice [381,382]. Furthermore, human APOE isoforms have been shown to modulate glucose and metabolic pathways, with the APOE ε3/ ε4 variants showing markedly reduced glucose

uptake and metabolism in mouse models [383]. APOE ε2 brains demonstrated a more robust metabolic profile than APOE ε3/ε4, suggesting a physiological mechanism for its protective role against AD [383]. Given human and animal model research for glucose metabolism playing a role in the AD biomarker continuum, we speculate that the relationship observed between a high-fat, high-sugar diet and increased cerebral Aβ deposition may be modulated by impaired glucose metabolism in this cohort. The results presented in this thesis suggest an impaired glucose metabolic pathway potentially being associated with an APOE-Aβ physiological mechanism. Furthermore; studies have shown that APOE ε4 confers a greater risk in women than men [365]. Women with a single APOE ε4 allele have up to a 4-fold increase in risk when compared to women homozygous for APOE ε3; however, men with a single APOE ε4 allele have little to no increase in risk [385]. Given animal model evidence for an APOE mediated glucose metabolism [383], females may experience greater AD risk due to a upstream mechanistic action in their glucose metabolism.

The findings relating a high fat, high sugar Junk Food diet to cerebral Aβ deposition in ageing Australian women strengthen the hypothesis of diet being a modifiable risk factor for AD. These results suggest that higher adherence to a high-fat, high-sugar style diet may be associated with an increased deposition of cerebral AD biomarkers and therefore a higher risk for developing this devastating disease.

7.5 Strengths

The strengths of this thesis are in the novel findings that have been reported in a number of understudied areas. The systematic review and meta-analysis substantiated evidence of a diet-AD interaction by providing qualitative and quantitative support for the notion that this relationship may be mediated by physiological biomarkers of the disease

At a greater risk of dementia and AD, there is a paucity of research investigating female-only cohorts. Representing a marginalised majority, there is a distinct lack of awareness of modifiable risk in women. This thesis was the first to relate dietary patterns to cerebral AD neuropathology in a female only cohort.

For follow-up, the same women attended assessments as part of the WHAP. Short-term follow-up dietary studies often assume dietary stability without capturing long term change therefore our study was strengthened by 14 years of follow-up dietary data on the same women Furthermore, the FFQ and method of nutrient calculation, collation and analysis were identical for both time points; therefore, reported changes in nutrient intakes are largely due to changes in portion size or frequency of consumption and will not be confused by changes in the dietary methodology.

Over 14 years of follow up, there were no significant differences in education, employment, age, BMI, smoking status, nutrient intake or MD adherence between the excluded and included participants in Chapter 6 (2012). This suggests that the loss to follow-up has not introduced a healthy bias into this thesis.

7.6 Limitations

Limitations are an unavoidable circumstance of research. The systematic review was limited by a small number of identified studies and their cross-sectional nature (14 out of 15) limited causal inferences from the available published findings. Given systematic review identified several studies pooled their cohorts from the same larger populations, the possibility of participants overlapping in separate studies may bias the strength the findings; however, all cohorts analysed in the systematic review were unique and were therefore not removed from qualitative analysis. Other biomarkers of neurodegeneration, such as white matter hyperintensities and atrophy may also contribute to the clinical

manifestation of disease progression; however, were outside the scope of the systematic review and meta-analysis presented in this thesis.

Individuals in the WHAP cohort are a select, Australian-specific population of women in the metropolitan area, which may impact upon the representability and the translatability of this work to the wider community. Participants were drawn from the same cohort pool based on data availability, potentially implicating a healthy cohort bias in the sample selected. Availability of data also impacted sample size for dietary pattern factor analysis (n = 183 in Chapter 5, n = 115 in Chapter 6), largely due to including only participants who agreed to undergo a PET scan in 2012. There is always the possibility of the results observed in this thesis being mediated by a confounding variable (medication, hypertension, uncontrolled diabetes) that was not included in the model. Statistical models were ultimately based on previous literature, data availability and distribution. In longitudinal analysis, healthy survivor bias is always a possibility; however, we saw predominantly non-significant differences in sociodemographic variables between the included cohort and those participants lost to follow-up.

The self-reported nature of several scales presented in this thesis is an additional limitation of the work. FFQs utilised in this thesis relied on a participant's estimation of intake over the preceding year, limiting the accuracy of dietary recall.

As has been previously discussed [328], our nutritional and dietary analysis was not able to account for changes in the composition of individual food items by manufacturers over time. This limits the accuracy of nutrient estimation from a given food item.

Other limitations of this study were the lack of socioeconomic status as a covariate and the small sample size. However, participant assessments are already intensive, invasive

and time-consuming and furthermore, this study is part of a longitudinal investigation over 25 years and drop out over this period of time is inevitable.

7.7 Future directions

For many decades, research investigating modifiable lifestyle risk factors has come under scrutiny for failing to provide evidence that interventions, not secondary factors, are responsible for AD risk reduction. Among the multitude of lifestyle factors we know about, the ones we don't know about and the interactions between these two, how is it ever possible to distinguish therapeutic interventions that mitigate AD risk? These questions point toward diet-AD epidemiologists to provide observational and interventional evidence that supports the theory that healthy dietary modification helps to prevent AD. In recent years, pharmacological disease-modifying therapies attracted the majority of research focus; however, have failed to present a drug with therapeutic potential. Multimodal lifestyle interventions that include diet as a protective factor; however, have continually provided support for the hypothesis presented in this thesis.

Further research is required to elucidate the sex-specific physiological mechanisms that underpin this relationship. Future research would benefit from a focus on dietary patterns with robust study designs and larger samples of males and females to clarify the therapeutic utility of diet in AD. The systematic review and meta-analysis presented in this thesis found only one longitudinal investigation that investigated dietary patterns and AD biomarkers at two distinct time points and only 3 studies that utilised an *a posteriori* method of dietary analysis. Future longitudinal research is of particular relevance to the development of preventative measures, as well-defined cohorts allow researchers to isolate mediators of a potential diet-AD mechanism. The findings presented in this thesis support a relationship between diet/nutrition and biomarkers of AD; however, given

biomarkers can manifest decades prior to symptomatology it remains to be seen how long-term dietary choices can impact subsequent pathology.

With additional funding, personnel and time, a second time point of amyloid PET imaging would provide a foundation for causal inference of a diet-AD relationship. Given amyloid takes decades to develop, a round of amyloid PET scans in the next 5 years (by 2024) would be able to provide substantial evidence for a causal mechanism. Given the diets of WHAP participants were seen to decline over time since their initial DQES v2 in 1998, it would be fascinating to elucidate the impact an increasingly unhealthy diet was having on amyloid accumulation. As returning WHAP participants have completed a DQES v2 at every time point since 2012, there would be substantial data to validate a claim of a diet-AD mechanism, particularly in those WHAP participants who completed the 2012 PET scan and would be interested in a follow up scan.

Furthermore, it would be illuminating to investigate the potential role of education and cognition in the diet-Aβ relationship in future epidemiological research. Given socioeconomic status may mediate the relationship between education, cognition and MeDi adherence due to an individual's ability to invest in healthier dietary choices, characterising the income level of participants may potentially explain some of the variation observed. Longitudinal cognitive data over the next several decades would provide support for the potential association of dietary adherence and cognitive decline. As WHAP participants risk of dementia continues to increase with ageing, future research could investigate this relationship with respect to dementia incidence/prevalence over time.

Education of evidence-based factors for dementia risk reduction is a crucial element of future research. Although research and dissemination are essential first steps, there is a responsibility for society to implement these interventions into practice. The 2017 *Lancet*

Commission on Dementia Prevention, Intervention and Care[13] notes these interventions must be accessible, sustainable and, if possible, enjoyable otherwise they will remain unutilised. Dietary guidelines for reducing an individual's risk of dementia must provide the hypothesis, the evidence and the actionable practices in order to foster uptake in the wider population.

7.8 Conclusions

Amidst epidemiological evidence that diet has substantial potential to be a therapeutic modifiable lifestyle risk factor for cognitive decline, this thesis investigated the prodromal neuropathological stage of AD. The main findings of this thesis indicate diet is related to the hallmark biomarkers of AD (Aβ and tau) that precede the clinical manifestation of AD. This thesis reported that diets are subject to change over an important period in a woman's life and this is consistently in the direction of a poorer diet. Dietary pattern adherence also had a significant impact on cerebral pathology in ageing Australian women. Consuming an unhealthy 'Junk Food' dietary pattern was a significant predictor of cerebral beta-amyloid accumulation.

In epidemiological research, the relationship between diet and brain health is complicated. However, technological advancements and discoveries in blood based biomarkers for AD are ushering in new methodologies of understanding disease progression and lifestyle factors that offer therapeutic potential. Smart phones are becoming ubiquitous in modern society and offer an accessible, efficient and low-cost mechanism of collecting dietary data, as well as a multitude of confounders such as education, physical activity, sleep and comorbidities. Harnessing the potential of epidemiological data collection through mobile phone application will be a challenge for future research, yet offers substantial potential to identify time-windows where modifiable lifestyle risk factors display the greatest therapeutic potential. AD poses a

significant economic, social and societal burden over the coming decades, with no pharmacological intervention displaying efficacious results. Without a cure in sight, research investigating the plethora of modifiable lifestyle risk factors that may alleviate cognitive decline, and the AD biomarkers that precede them, is a research necessity.

In the next decade or so, AD will become the leading cause of death in Australia. To alleviate the potential burden placed on millions of Australians, their partners, loved ones and carers, recommendations for the uptake of healthy lifestyle behaviours are essential. It is increasingly clear that the evidence for a panacea to treat AD is a pipedream. One scenario more likely is the potential of lifestyle modification based upon individual circumstance to alleviate AD burden. A precision medicine approach based upon genetic, sex, age and lifestyle factors may offer the best potential to alleviate AD. Through international collaborations, data linkage and cross-cohort studies, the effectiveness of a precision medicine approach can be tested through recommending continuing education, dietary adherence, physical activity, sleep hygiene or other factors that have therapeutic potential for an individual at risk for AD.

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9 APPENDICES

The purpose of the following appendices is to provide supplementary material to aid in the comprehension of this thesis. Participant informed consent, scale measures, conference presentations and published paper author contributions will be presented herein.

APPENDIX 1: PARTICIPANT INFORMED CONSENT FORM 2012

Women's Healthy Ageing Study

The University of Melbourne

Participant Information and Consent Form

Version 11:6 26th November, 2013

Site: The University of Melbourne

Full Study Title: Women's Healthy Ageing Project (WHAP)

Director: Associate Professor Cassandra Szoeke

Associate Researchers: Professor David Ames, Dr Kathryn Ellis, Associate Professor Cassandra Szoeke, Professor Lorraine Dennerstein.

This Participant Information and Consent Form is 11 pages long. Please make sure you have all the pages.

1. YOUR CONSENT

Thank you for your continued interest in volunteering to participate in research. You are invited to take part in this research study on ageing.

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. 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 STUDY

Whilst studies across the menopausal transition are prevalent, there is very little information about the natural history of cardiovascular, bone and mental health functioning after the final

menstrual period, and how these are affected by a range of factors including premenopausal characteristics.

There is also scant knowledge about changes in symptoms, mood, sexuality and other aspects of quality of life in the time following menopause. This knowledge may allow the early identification of women who are at risk of adverse changes as well as clarifying the role of hormones and identifying modifiable factors amenable via interventions that could improve the health status of postmenopausal women in the future.

This study plans to investigate if hormonal, lifestyle, behavioural and genetic factors are associated with memory and changes to your brain. Participation involves having a blood test, and also involves using non-invasive tests to assess your memory. The tests will include measurements of short and long-term verbal memory, non-verbal memory and concentration tasks which are a measure of 'working memory'. These measurements will then be linked to the data we have collected annually over the past 11 years and will collect at the time of the memory tests [ie. lifestyle, behavioural (stress, daily hassles, paid work), blood pressure, and hormonal factors (menopausal status and hormone therapy use)]. These measures will then be analysed to determine whether any factors are associated with these memory scores.

If you decide to participate in the study, you will also be invited to have Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scans of your brain. These brain scans are an optional part of the study.

3. PROCEDURES

This study involves two visits:

Visit 1 will be a baseline visit. Visit 2 will be a follow-up visit 18 months later.
In addition to these visits, we seek your permission to contact you to give you information or updates about the study.

Each visit will involve a session lasting approximately 4 hours by a trained member of staff on a single morning. As the sessions run for an extended period of time, opportunities will be provided for rest and refreshment breaks as required. During these visits, a physical examination will be performed including a blood test, blood pressure, heart rate, weight and your height, as an assessment of your overall health. This component of the study takes approximately 45 minutes.

At both visits, you will be asked to complete some memory and attention tasks. These will help the researcher understand how well your memory is working. These tasks will take approximately 1.5 hours.

You will be asked about your medical history, and about any medications you are currently using and/or have used in the past 4 months, including prescription (medications your doctor has prescribed) and over the counter medications (medications, vitamins, and other remedies you may buy). You will be asked about your mood and behaviour, as this helps the researcher understand if you may be at risk of depression.

If you are thought to be at risk for depression, we will seek your permission to discuss this with your General Practitioner so that you may obtain treatment. This will not mean you cannot participate in the study.

You will also be asked to complete two questionnaires about your diet and exercise levels, a short questionnaire about your memory, and two questionnaires about your interpersonal relationships. In addition, a staff member will ask you questions about your demographic information, lifestyle, family history, mood, and stress levels.You will be provided with a short questionnaire asking whether you have noticed any changes regarding your ability to remember. You will also be provided with a brief personality questionnaire. Some of these questionnaires will be mailed out to you to complete before the day, and others will be completed with a staff member during your assessment. The questionnaires completed on the day of your appointment will take about an hour.

With regard to the blood test, 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 16 teaspoons.

Your blood 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 the researcher and members of the research team to identify your results.

One of the reasons that we collect a sample of your blood is to investigate your genetic material, deoxyribonucleic acid (DNA). You have the option of instructing us on how your DNA is to be used and stored. If you agree to participate in this study and when you sign the consent form, you will be asked to select one of three options for using and storing your DNA. Irrespective of which option you choose, your data will remain confidential and de-identified at all times.

1. Discard my DNA sample after it has been tested for the specific purpose of this study.
2. Test and then store my DNA sample indefinitely for future research in the field of healthy ageing.
3. Test and then store my DNA sample indefinitely for future unspecified research.

Medical science is advancing very quickly, and so we are not in a position to be able to tell you exactly what form the future research might take, or the consequences of that research. Future research might involve isolating biological markers for disease, or ascertaining the effects of lifestyle influences and different drugs on the WHAP cohort. This is the type of unspecified research referred to in point 3, above.

This research might lead to drugs or tests that are produced and marketed by private organisations for profit. The WHAP study may or may not benefit from any of the revenue that such research may produce and if the research does lead to discoveries that are of commercial value to the researchers and their institutions, there will be no financial benefit to yourself or your family.

No research will take place using your blood or DNA samples or any other information that you provide unless that research is first reviewed and approved by a properly constituted Human Research Ethics Committee, which will determine whether the benefits of the research outweigh the cost to you and your privacy.

As part of your participation in the Women's Healthy Ageing Study, you will be invited to receive a Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scan

of your brain following each assessment. This component of the study is optional. MRI scans provide information about the structure of your brain, whilst PET scans provide information about how it is functioning. If you agree to having the brain scans, these will be booked on separate days. MRI scans are done at the Royal Melbourne Hospital (Parkville), and PET scans are done at the Melbourne Brain Centre on Royal Parade, Parkville.

MRI scan

The MRI scan will require you to lie still in a scanner for 20 minutes. There are no injections involved in this scan.

PET scan

Before each scan, one small plastic needle will be inserted into a vein in your arm. This will be used to inject the small dose of radioactive labelled marker which will mix with your blood to travel to your brain. Thirty minutes later you will be asked to lie in the PET scanner while a scan of your brain is being taken. The whole process takes about an hour and a half (counting the waiting time between your injection and lying under the camera).

4. BENEFITS

You will not receive any direct benefit from participating in this 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 ageing.

The major findings from this study are being used to provide:

· Women with accurate information about normal experiences of healthy ageing;

· Healthcare professionals with information about the problems which concern mid-aged women;

· Information to the Australian Government and other international bodies for use in formulating policies on healthcare and practice guidelines;

· Information for planning clinical trials and future research into chronic diseases and ageing.

5. RISKS, DISCOMFORTS, AND RISK MANAGEMENT

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 extremely rare 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, you will be provided with an opportunity to eat before further testing is done, and you will be reimbursed for

the cost of the food. To minimise the risks associated with your blood test, all equipment is contained in single-use sterile packaging, opened only immediately before your test is performed. Staff taking your blood are all appropriately qualified and experienced to do so. You will sit in a comfortable, specially-designed chair to have your blood taken, or are able to lie down on a bed if you prefer.

Risks associated with cognitive tasks

You may experience anxiety or psychological discomfort while completing the memory assessment. All researchers completing the memory assessment are trained in psychology and will be sensitive to how you are feeling throughout the tasks. If you no longer wish to continue your participation at any stage, the assessment can be discontinued immediately.

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 investigator's office and electronic copies stored on file with password restricted access.

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

If you choose to take part in the optional brain imaging componenet of the study, you will attend separate appointments at the Royal Melbourne Hospital and Melbourne Brain Centre for these scans, and sign separate consent forms for each of them. The brain scans have been approved by the Austin Health Ethics Committee (HREC number: H2011/04354).

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 tell your study team if you have any metal, metal devices, or electronics in your body. The study staff will go through a checklist with you. For the scan, you will have to lie still on your back for about 20 minutes in the MRI scanner, which is a small space. This may be difficult if you are claustrophobic. Some MRI machines are noisy and you may find this discomforting. You will be able to talk to hospital staff throughout the scan, and it can be stopped at any time at your request.

Risks associated with PET scans

The PET scan involves having a catheter inserted into a vein in your arm, to give you an injection. Risks associated with a venous catheter are slight pain and bruising at the site of the injection, and an extremely rare chance of infection.. The scans also involve exposure to a small amount of a radioactive compound called Florobetaben. 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 total effective dose from these scans is 3.7 mSv. This is typical of the dose received from many other diagnostic medical x-ray and nuclear medicine procedures. At this

dose, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The additional risk of an induced fatal cancer is believed to be low and theoretically is approximately equivalent to 1 in 4,200.

Additional possible risks

The possibility exists for a rare reaction to any of the procedures to which the participant will be exposed. In the event that you become uncomfortable, anxious, or distressed during any part of your participation in the study, it will be possible to take a rest break or discontinue your involvement if you wish. Additionally, the researcher will be able to provide you with referral options to psychological support services if required.

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.

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 only be used for the purpose of this study. Your DNA sample will either be destroyed or stored for use in this or other studies, depending on whether you give your consent for it to be stored for future use (see Section 3, above). The information will be securely stored in a locked filing cabinet and access to the data will be limited to individuals working directly on this project. All computer files will link data from individuals by a code number. It will only be disclosed with your permission, 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 participant will be identifiable by name in any publications/presentations arising from the study.

In accordance with The Freedom of Information Act 1982 (Vic) and other relevant health records and privacy laws, you can request to have access to information collected about you during the study, including your medical records.

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.

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.

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 or your carer (if applicable) a brief verbal or written summary of the overall results of the study upon request.

10. DATA LINKAGE

As the Women's Healthy Ageing Project is a longitudinal study over twenty years, its advantage is in helping us observe your health over time, and track factors that have influenced your health across your lifespan. Great yield can be achieved by linking your information provided to us in questionnaires and study tests with information about your medical history from a variety of healthcare providers, such as doctors and hospitals. This includes information about your past, current and future health, medication, treatment and use of health services, which may be obtained from your medical records, as well with data from other similar studies that you advise us you are participating or have participated in. By signing the attached Consent Form, you authorise release of, or access to, your medical history (both past and into the future). Any information obtained through this process will be de-identified by our research team and kept confidential, as per all other information about you collected in the study.

We also request your permission to allow us to share your data with other researchers who are currently, or in the future, conducting research studies similar to this one. Again, any information about you provided to other researchers will not include any identifying features, and will be provided only to studies who have sought appropriate clearance from relevant Human Research Ethics Commitees.

If you choose not to participate in this additional element of the study, it will not influence your participation in the rest of the study.

11. 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, A/Prof Cassandra Szoeke on (03) 8344 1835, or our Research Officer (responsible for participant liaison) Jacqueline Giummarra on (03) 9389 2958.

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

Name: Kate Murphy

Position: Research Manager at The University of Melbourne.

Telephone: (03) 8344 2073

You will need to tell the Patient Representative that Professor Ames and Associate Professor Cassandra Szoeke are the Principal Investigators.

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, and any unprocessed data will be destroyed.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your pariticipation or your relationship with us.

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 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 before you withdraw.

13. ETHICAL GUIDELINES

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)* which 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 Health Sciences Human Research Ethics Committee of The University of Melbourne.

15. REIMBURSMENT

There will be no payments made to participants in this study. All study participants are eligible for reimbursement of transportation costs incurred by travelling to and from clinic appointments, and the cost of food if you are required to fast before a blood test. Participants will incur no expense for any tests or procedures that are undertaken for study purposes.

Women's Healthy Ageing Study

The University of Melbourne

Participant Consent Form

Version 11.6: 11th November, 2013

Site: The University of Melbourne

Full Study Title: Women's Healthy Ageing Project (WHAP)

- 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 in the Participant Information.
- I will be given a copy of the Participant Information and Consent Form to keep.
- 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.

Optional parts of the research project; please CIRCLE your response:

- I may be contacted between visits to provide additional information or receive study updates YES NO
- I give my consent for my data to be stored indefinitely for future research YES NO
- YES NO
- I give my consent to data linkage being used to link my study data to additional sources of my health information YES NO
- I give my consent to share my data with any identifying features removed with other researchers YES NO
- I give my consent to be contacted about participation in future studies YES NO

Duration of Storage of my DNA Sample; Tick **ONLY ONE box**:

☐ I give my consent to the testing and then storage of my DNA sample for research in the field of healthy ageing.

☐ I consent to the testing and then storage of my DNA sample for future unspecified research

☐ I consent to the testing of my DNA sample for the specific purpose of this study on the condition that my DNA sample is discarded immediately thereafter

Participant's Name
(printed)_____

Signature_____Date_____
____

Name of Witness
(printed)_____

Signature_____
Date_____

Researcher'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.

*A senior member of the research team (who is a registered medical practitioner) must provide the explanation and provision of information concerning the research project.

Note: All parties signing the Consent Form must date their own signature.

Women's Healthy Ageing Study

The University of Melbourne

RELEASE OF MEDICAL INFORMATION

Date: _____

By ticking the boxes and signing below, I_____
(print name)

☐ Consent to the WHAP team releasing my results from this study to my GP and/or specialist doctor _____ .
(print name)

☐ Consent to the WHAP teams releasing relevant (deidentified) information from this study to other researchers

☐ Consent to the release of my medical records*, both past and into the future, to the WHAP study team

*Medical information may include your medical history, prescribed medications, hospital

admission/progress notes, specialist results and reports, and other similar medical records.

Signature: _____

Women's Healthy Ageing Study

The University of Melbourne

Participant Revocation of Consent Form

Version 11.6: 11th November, 2013

Site: The University of Melbourne

Full Study Title: Women's Healthy Ageing Project (WHAP)

I hereby wish to WITHDRAW my consent to participate in the research proposal named above and understand that such withdrawal WILL NOT jeopardize my relationship with The University of Melbourne.

Participant's Name (printed)_____

Signature: _____Date_____

APPENDIX 2: PARTICIPANT PLAIN LANGUAGE STATEMENT

National Ageing Research Institute

Participant Information and Consent Form

Version 11:4 12th April, 2011

Site: National Ageing Research Institute

Full Study Title: Women's Healthy Ageing Project (WHAP)

Director: Associate Professor Cassandra Szoeke

Associate Researchers: Professor David Ames, Dr Kathryn Ellis, Associate Professor Cassandra Szoeke, Professor Lorraine Dennerstein.

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

1. YOUR CONSENT

You are invited to take part in this research study on ageing.

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. 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 STUDY

The Australian population is ageing at a dramatic rate. The Australian Bureau of Statistics reported that in 2001, 12.4% of the population was aged over 65 years, with only 3% aged 80 years or older. It is predicted that in 2051, 26.1% of the population will be older than 65 years and 9.4% 80 years or older (Australian Bureau of Statistics,

2001). The majority of people aged over 85 - almost 70% - are women (Department of Health and Ageing, 2000).

Old age increases the risk for physical health problems, especially cardiovascular disease, and neurodegenerative disease such as Dementia; and in those over the age of 75, the presence of more than one disease is common. Major causes of illnesses and death in postmenopausal women include cardiovascular disease, depression and bone health. These have all been linked to menopausal hormonal changes and large clinical trials of various forms of hormone therapy have shown different results.

While many studies have been conducted on the menopausal transition, there is very little information about cardiovascular, bone and mental health functioning after the final menstrual period and how these are affected by a range of factors including premenopausal characteristics.

This knowledge may allow the early identification of women who are at risk of unfavourable cognitive changes after the final menstruation period as well as clarifying the role of hormones and identifying factors that could be targeted to improve the health status of postmenopausal women in the future. There is also limited knowledge about changes in symptoms, mood, sexuality and other aspects of quality of life in the immediate post menopause.

This study plans to investigate if hormonal, lifestyle, behavioural and genetic factors are associated with memory. The study involves using non-invasive tests to assess your memory. The tests will include measurements of short and long-term verbal memory, non-verbal memory and concentration tasks which are a measure of ‘working memory’. These measurements will then be linked to the data we have collected annually over the past 11 years and will collect at the time of the memory tests [ie. lifestyle, behavioural (stress, daily hassles, paid work), blood pressure, and hormonal factors (menopausal status and hormone therapy use)]. These measures will then be analysed to determine whether any factors are associated with these memory scores.

3. PROCEDURES

This study involves two visits:

Visit 1 will be a baseline visit. Visit 2 will be a follow-up visit 18 months later.

At both these visits, you will be required to perform some cognitive tasks and have a blood test.

These two visits will involve a session lasting approximately 4 hours by a trained member of staff on a single morning. The risks involved in these investigations are

minimal. All information provided is treated as confidential and securely stored. All documents with identifying information will be stored in a locked filing cabinet and access to the data will be limited to individuals working directly on this project. All computer files will link data from individuals by a code number. Research data from this study will be published without names and in such a way that identification of individuals is not possible. All data will be destroyed 10 years after publication of the results.

During these visits, a physical examination will be performed including blood pressure, heart rate, weight and your height, as an assessment of your overall health.

You will be asked about your medical history, and about any medications you are currently using and/or have used in the past 4 months, including prescription (medications your doctor has prescribed) and over the counter medications (medications, vitamins, and other remedies you may buy). You will be asked about your mood and behaviour, as this helps the study doctor understand if you may be at risk of depression.

At both visits, you will be asked to complete some memory and attention tasks. These will help the doctor understand how well your memory is working.

You will also be asked to complete two questionnaires about your diet and exercise levels.

You will be provided with a short questionnaire asking whether you have noticed any changes regarding your ability to remember. You will also be provided with a brief personality questionnaire.

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 16 teaspoons.

One of the reasons that we collect a sample of your blood is to investigate your genetic material, deoxyribonucleic acid (DNA). You have the option of instructing us on how your DNA is to be used and stored. If you agree to participate in this study and when you sign the consent form, you will be asked to select one of three options for using and storing your DNA.

1. Discard my DNA sample after it has been tested for the specific purpose of this study.

2. Test and then store my DNA sample for a period of ten years after results have been published for future research in the field of Alzheimer's disease.

3. Test and then store my DNA sample for a period of ten years after results have been published for future unspecified research.

Your blood 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.

You will also be provided with a booklet which includes a detailed history of the study, including previous findings and information on the current study.

4. BENEFITS

You will not receive any direct benefit from participating in this 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 ageing.

The major findings from this study are being used to provide:

· women with accurate information about normal experiences of healthy ageing;

· healthcare professionals with information about the problems which concern mid-aged women;

· information to the Australian Government and other international bodies for use in formulating policies on healthcare and practice guidelines;

· information for planning clinical trials and future research into chronic diseases and ageing.

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 extremely rare 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, you will be provided with an opportunity to eat before further testing is done, and you will be reimbursed for the cost of the food.

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 investigators office and electronic copies stored on file with password restricted access.

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.

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 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 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.

In accordance with The Freedom of Information Act 1982 (Vic) and other relevant health records and privacy laws, you can request to have access to information collected about you during the study, including your medical records.

Members of the research team may examine your health records if you give permission for them to do so. 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.

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.

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 investigators will provide you or your carer (if applicable) 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 or the study coordinator. The researchers responsible for this study are the principal investigators, Professor Ames, Ph: 03 9272 0485 and Associate Professor Cassandra Szoeke, Ph: 03 9387 2520 and the study coordinator, Dr Kathryn Ellis, Ph: 03 9272 0436.

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

Name: Kate Murphy

Position: Executive Officer Research at The University of Melbourne.

Telephone: (03) 8344 2073

You will need to tell the Patient Representative that Professor Ames and Associate Professor Cassandra Szoeke are the Principal Investigators.

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 and the medical clinic/hospital.

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 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 before you withdraw.

13. ETHICAL GUIDELINES

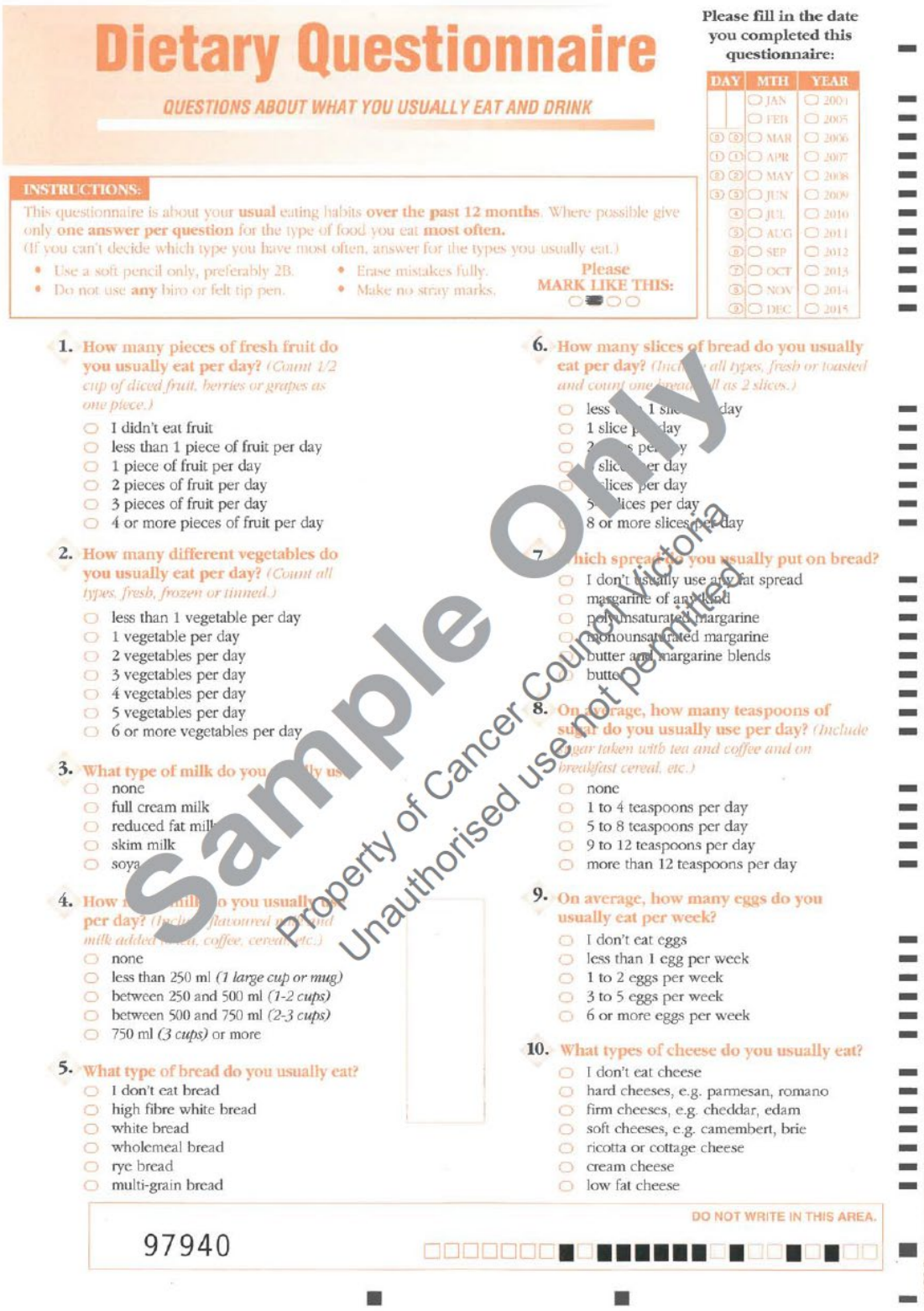
This project will be carried out according to the National Statement on Ethical Conduct in Research Involving Humans (2007) produced by the National Health and Medical Research Council of Australia, the Australian Research Council and Australia Vice-Chancellor's Committee. This statement has been developed 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 Human Research Ethics Committee of The University of Melbourne.

14. REIMBURSMENT

There will be no payments made to participants in this study. All study participants are eligible for reimbursement of transportation costs incurred by travelling to and from clinic appointments. Food will be provided to you if you are required to fast before a blood test. Participants will incur no expense for any tests or procedures that are undertaken for study purposes.

APPENDIX 3: DIETARY QUESTIONNAIRE FOR EPIDEMIOLOGICAL STUDIES VERSION 2



Dietary Questionnaire

QUESTIONS ABOUT WHAT YOU USUALLY EAT AND DRINK

Please fill in the date you completed this questionnaire:

DAY	MTH	YEAR
	○ JAN	○ 2004
	○ FEB	○ 2005
⓪ ⓪	○ MAR	○ 2006
① ①	○ APR	○ 2007
② ②	○ MAY	○ 2008
③ ⑤	○ JUN	○ 2009
	④ ○ JUL	○ 2010
	⑤ ○ AUG	○ 2011
	⑥ ○ SEP	○ 2012
	⑦ ○ OCT	○ 2013
	⑧ ○ NOV	○ 2014
	⑨ ○ DEC	○ 2015

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often.**
(If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ○■○○

1. How many pieces of fresh fruit do you usually eat per day? *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*

- ○ I didn't eat fruit
- ○ less than 1 piece of fruit per day
- ○ 1 piece of fruit per day
- ○ 2 pieces of fruit per day
- ○ 3 pieces of fruit per day
- ○ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? *(Count all types, fresh, frozen or tinned.)*

- ○ less than 1 vegetable per day
- ○ 1 vegetable per day
- ○ 2 vegetables per day
- ○ 3 vegetables per day
- ○ 4 vegetables per day
- ○ 5 vegetables per day
- ○ 6 or more vegetables per day

3. What type of milk do you [illegible]

- ○ none
- ○ full cream milk
- ○ reduced fat milk
- ○ skim milk
- ○ soya

4. How [illegible] milk do you usually [illegible] per day? *(Include flavoured milk and milk added to tea, coffee, cereal, etc.)*

- ○ none
- ○ less than 250 ml *(1 large cup or mug)*
- ○ between 250 and 500 ml *(1-2 cups)*
- ○ between 500 and 750 ml *(2-3 cups)*
- ○ 750 ml *(3 cups)* or more

5. What type of bread do you usually eat?

- ○ I don't eat bread
- ○ high fibre white bread
- ○ white bread
- ○ wholemeal bread
- ○ rye bread
- ○ multi-grain bread

6. How many slices of bread do you usually eat per day? *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*

- ○ less [illegible] 1 slice per day
- ○ 1 slice per day
- ○ 2 [illegible]s per day
- ○ [illegible] slices per day
- ○ [illegible]lices per day
- ○ [illegible]lices per day
- ○ 8 or more slices per day

7. [illegible]hich spreads do you usually put on bread?

- ○ I don't usually use any fat spread
- ○ margarine of any kind
- ○ polyunsaturated margarine
- ○ monounsaturated margarine
- ○ butter and margarine blends
- ○ butter

8. On average, how many teaspoons of sugar do you usually use per day? *(Include sugar taken with tea and coffee and on breakfast cereal, etc.)*

- ○ none
- ○ 1 to 4 teaspoons per day
- ○ 5 to 8 teaspoons per day
- ○ 9 to 12 teaspoons per day
- ○ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ○ I don't eat eggs
- ○ less than 1 egg per week
- ○ 1 to 2 eggs per week
- ○ 3 to 5 eggs per week
- ○ 6 or more eggs per week

10. What types of cheese do you usually eat?

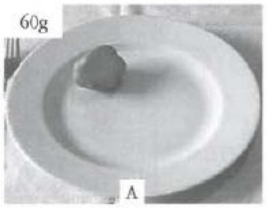
- ○ I don't eat cheese
- ○ hard cheeses, e.g. parmesan, romano
- ○ firm cheeses, e.g. cheddar, edam
- ○ soft cheeses, e.g. camembert, brie
- ○ ricotta or cottage cheese
- ○ cream cheese
- ○ low fat cheese

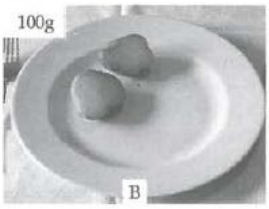
DO NOT WRITE IN THIS AREA.

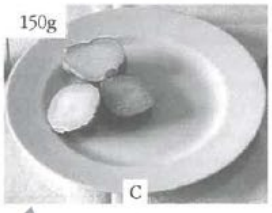
97940

For each food shown on this page, indicate ***how much on average you would usually have eaten at main meals during the past 12 months.*** *When answering each question, think of the* ***amount*** *of that food you usually ate, even though you may rarely have eaten the food on its own.*
If you usually ate more than one helping, fill in the oval for the serving size closest to the ***total amount*** *you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato

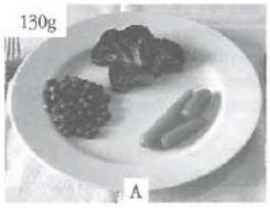


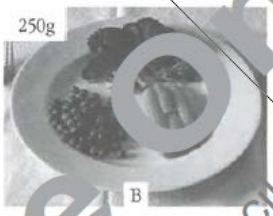


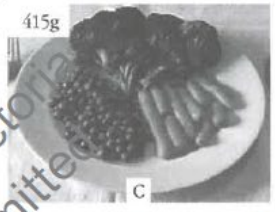


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables

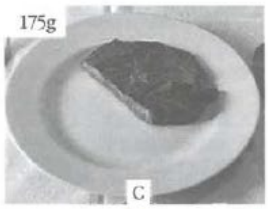






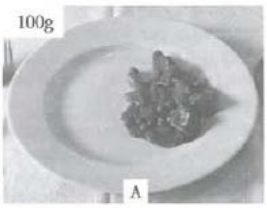
○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

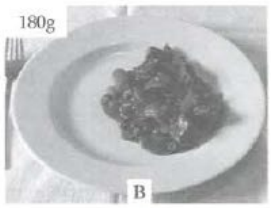
13. When you ate steak, did you usually eat: ○ I never ate steak

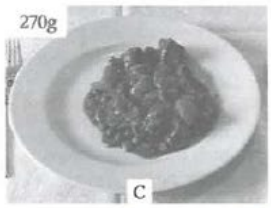


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole







○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. Over the last 12 months, on average, *how often* did you eat the following foods? *Please completely fill one oval in every line.* Please MARK LIKE THIS: ● NOT LIKE THIS: ✓ ✗ —

Times You Have Eaten		NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month		per week				per day		
CEREAL FOODS, SWEETS & SNACKS											
All Bran™	A1	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™	A2	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™	A3	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™	A4	○	○	○	○	○	○	○	○	○	○
Porridge	A5	○	○	○	○	○	○	○	○	○	○
Muesli	A6	○	○	○	○	○	○	○	○	○	○
Rice	A7	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne)	A8	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits	A9	○	○	○	○	○	○	○	○	○	○
Sweet biscuits	A10	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries	A11	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries	A12	○	○	○	○	○	○	○	○	○	○
Pizza	A13	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun	A14	○	○	○	○	○	○	○	○	○	○
Chocolate	A15	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™, etc.)	A16	○	○	○	○	○	○	○	○	○	○
Nuts	A17	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste	A18	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™, etc.	A19	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups	A20	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™	A21	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH											
[illegible]	B1	○	○	○	○	○	○	○	○	○	○
Ice-[illegible]m	B2	○	○	○	○	○	○	○	○	○	○
[illegible]ogh[illegible]rt	B3	○	○	○	○	○	○	○	○	○	○
Beef	B4	○	○	○	○	○	○	○	○	○	○
Veal	B5	○	○	○	○	○	○	○	○	○	○
Chicken	B6	○	○	○	○	○	○	○	○	○	○
Lamb	B7	○	○	○	○	○	○	○	○	○	○
Pork	B8	○	○	○	○	○	○	○	○	○	○
Bacon	B9	○	○	○	○	○	○	○	○	○	○
Ham	B10	○	○	○	○	○	○	○	○	○	○
[illegible]ne[illegible]f, luncheon meats or salami	B11	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	B12	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	B13	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	B14	○	○	○	○	○	○	○	○	○	○
[illegible], tinned (salmon, tuna, sardines, etc.)	B15	○	○	○	○	○	○	○	○	○	○
FRUIT											
Tinned or frozen fruit (any kind)	C1	○	○	○	○	○	○	○	○	○	○
Fruit juice	C2	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit	C3	○	○	○	○	○	○	○	○	○	○
Apples	C4	○	○	○	○	○	○	○	○	○	○
Pears	C5	○	○	○	○	○	○	○	○	○	○
Bananas	C6	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew, etc.	C7	○	○	○	○	○	○	○	○	○	○
Pineapple	C8	○	○	○	○	○	○	○	○	○	○
Strawberries	C9	○	○	○	○	○	○	○	○	○	○
Apricots	C10	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines	C11	○	○	○	○	○	○	○	○	○	○
Mango or paw paw	C12	○	○	○	○	○	○	○	○	○	○
Avocado	C13	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten CONTINUED

	NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)										
Potatoes, roasted or fried (include hot chips)	D1	○	○	○	○	○	○	○	○	○
Potatoes cooked without fat	D2	○	○	○	○	○	○	○	○	○
Tomato sauce, tomato paste or dried tomatoes	D3	○	○	○	○	○	○	○	○	○
Fresh or tinned tomatoes	D4	○	○	○	○	○	○	○	○	○
Peppers (capsicum)	D5	○	○	○	○	○	○	○	○	○
Lettuce, endive, or other salad greens	D6	○	○	○	○	○	○	○	○	○
Cucumber	D7	○	○	○	○	○	○	○	○	○
Celery	D8	○	○	○	○	○	○	○	○	○
Beetroot	D9	○	○	○	○	○	○	○	○	○
Carrots	D10	○	○	○	○	○	○	○	○	○
Cabbage or Brussels sprouts	D11	○	○	○	○	○	○	○	○	○
Cauliflower	D12	○	○	○	○	○	○	○	○	○
Broccoli	D13	○	○	○	○	○	○	○	○	○
Silverbeet or spinach	D14	○	○	○	○	○	○	○	○	○
Peas	D15	○	○	○	○	○	○	○	○	○
Green beans	D16	○	○	○	○	○	○	○	○	○
Bean sprouts or alfalfa sprouts	D17	○	○	○	○	○	○	○	○	○
Baked beans		○	○	○	○	○	○	○	○	○
Soy beans, soy bean curd or tofu	D19	○	○	○	○	○	○	○	○	○
Other beans (include chick peas, lentils, etc.)	D20	○	○	○	○	○	○	○	○	○
Pumpkin	D21	○	○	○	○	○	○	○	○	○
Onion or leeks	D22	○	○	○	○	○	○	○	○	○
Garlic (not garlic t	D23	○	○	○	○	○	○	○	○	○
Mu ro	D24	○	○	○	○	○	○	○	○	○
hir	D25	○	○	○	○	○	○	○	○	○

16. **Over the last 12 months, how often did you drink beer, wine and/or spirits?**

Times That You Drank

	NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)	1	○	○	○	○	○	○	○	○	○
Beer (full strength)	2	○	○	○	○	○	○	○	○	○
Red wine	3	○	○	○	○	○	○	○	○	○
hite ne (include sparkling wines)	4	○	○	○	○	○	○	○	○	○
Fortified wines (port, sherry etc.	5	○	○	○	○	○	○	○	○	○
Spirits, liqueurs, etc.	6	○	○	○	○	○	○	○	○	○

the next two questions, please convert the amounts you drank into glasses using the examples given below. For spirits, liqueurs and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 stubby of beer = 1 glass
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. **Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?**

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	○	○	○	○	○	○	○	○	○	○

18. **Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?**

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	○	○	○	○	○	○	○	○	○	○

Thank You for completing this questionnaire

DO NOT WRITE IN THIS AREA.

97940

4

APPENDIX 4: CONSORTIUM TO ESTABLISH A REGISTRY FOR ALZHEIMER'S DISEASE

Memory Assessment

10 Item Supraspan Word List Recall Task (CERAD) – Delayed Recall Task

Instructions

I am going to read you a list of ten words. When I am finished reading the list I want you to repeat back as many of the words as you can remember.

WORD	**1**	**2**	**3**	**Delayed Recall**
DISH				
JESTER				
HILL				
COAT				
TOOL				
FOREST				
WATER				
LADDER				
GIRL				
FOOT				
TOTAL				

Trial 1 – Forwards (DISH – FOOT)

Trial 2 – Backwards (FOOT – DISH)

Trial 3 – Forest down through to TOOL

Delayed Recall at 3 minutes

APPENDIX 5: POSTER PRESENTATIONS

2017 The 13th International Conference on Alzheimer's & Parkinson's Disease, Vienna, Austria: The Role of Individual Mediterranean Diet Components on Beta-Amyloid Deposition in Ageing Australian Women

The Role of Individual Mediterranean Diet Components on Beta-Amyloid Deposition in Ageing Australian Women

Edward Hill[1], Cassandra Szoeke[2]

[1,2] Women's Heathy Ageing Project, University of Melbourne, Royal Melbourne Hospital, Institute for Health & Ageing, Australian Catholic University

Introduction

- Alzheimer's disease (AD) is the most common cause of dementia, accounting for approximately 60–80% all cases[1]
- By 2022, AD and dementia will overtake ischaemic heart disease as the leading cause of death in Australia[2]
- Distinct lack of efficacious pharmaceuticals for AD/dementia[3]
- 2017 Alzheimer's Association report confirmed that population-based studies found modifiable risk factors, such as diet, display therapeutic potential[1]
- Research is shifting towards modifiable lifestyle risk factors that display neuroprotective effect
- Mediterranean Diet (MeDi) has shown promising outcomes for cognitive health [4,5] and AD risk [6]
- The MeDi is a traditional dietary pattern characterised by a high intake of vegetables, legumes, fruits and cereals, a moderate intake of fish and dairy and a low intake of meat and poultry

Very few studies have investigated the role of MeDi adherence on AD biomarkers such as beta-amyloid (βA). βA plaques arise from the β-secretase cleavage of an integral membrane protein amyloid precursor protein. The current amyloid hypothesis states that the progression of these insoluble βA plaques around neurons and synaptic junctions causes neurodegeneration and subsequent dysfunction[7]. No study has investigated the effect of individual MeDi component adherence on (βA), a hallmark pathophysiological biomarker of AD. Furthermore, very few studies investigate female cohorts, who are at a greater risk for the disease due to genotypic/environmental factors.

Images courtesy of National Institute of Health[1] - Preventing Alzheimer's Disease: What do we know?

Aim

- To examine the impact of individual Mediterranean dietary components on beta-amyloid deposition in a cohort of ageing Australian women

Method

- 111 healthy ageing Australian women
- Dietary Questionnaire (DQESv2) responses used to calculate MeDi score
- PET imaging for βA accumulation

Participants were from the 2012 follow-up of the Women's Healthy Ageing Project (WHAP), a prospective longitudinal study of healthy Australian women.

Participants completed a validated food frequency questionnaire and MeDi scores were calculated from nutritional intake using a literature based scoring method [8]. βA deposition was measured via *in vivo* F-18 Florbetaben positron emission tomography scanning in 2012 and standard uptake value ratios (SUVR) were generated by normalising regional SUVs.

Participants age, education and BMI were collected during assessments. Cognition was measured using a comprehensive neuropsychological battery. Genotyping was conducted to determine presence of the APOE-ε4 allele. Complete data was available for 111 of the 124 scanned participants. All analyses were adjusted for covariates (age, education, BMI, energy intake and cognition.

Results

Figure 1. Characteristics of the cohort included in analysis (n = 111)

	Mean ± SD (n = 111)
Age (in years)	69.7 ± 2.5
Education (in years)	12.9 ± 3.7
Energy (kJ/day)	5286 ± 1723.4
BMI	28.3 ± 5.4
APOE-ε4 Positive n (%)	37 (33.3%)
PET SUVR Index	1.1295 ± 0.2
MeDi Adherence	5.8 ± 1.9

Characteristics of the cohort are displayed in Figure 1. PET SUVR Index distribution displayed a highly positively skewed distribution that was not rectified by multiple square root or log natural transformations. Inspection of P-P plots and AIC/BIC illustrated a generalised gamma linear model was best suited to PET SUVR distribution. Results of the gamma regression are displayed in Figure 2. Two MeDi components (legume & cereal) displayed significance, however these effects sizes were minimal.

*Figure 2. Gamma regression coefficient table for individual MeDi components (IV) and PET SUVR Index (DV) (*Significant at p<0.05)*

	Generalized Gamma Regression Coefficient Table			
	Beta	Sig.	CI Lower	CI Upper
Vegetable Intake (g/day)	0.000	0.332	-0.001	0.000
Legume Intake (g/day)	0.000	0.036*	0.000	0.001
Fruit & Nut Intake (g/day)	0.000	0.220	0.000	0.000
Dairy Intake (g/day)	0.000	0.303	0.000	0.000
Cereal Intake (g/day)	0.000	0.041*	0.000	0.001
Meat Intake (g/day)	-0.001	0.319	-0.002	0.001
Fish Intake (g/day)	0.000	0.664	-0.001	0.001
MUFA:SF Ratio	-0.044	0.506	-0.174	0.086
Alcohol Intake (g/day)	0.000	0.493	-0.002	0.001

Discussion

MeDi adherence was not significantly associated with beta-amyloid deposition (p>0.05) after adjusting for covariates. Of the MeDi components, greater cereal and legume intake was significantly associated with decreased beta-amyloid deposition (p<0.05) after adjusting for covariates. All other MeDi components (vegetable, fruit & nut, dairy, meat, fish, MUFA:SF and alcohol) were not significantly associated with beta-amyloid deposition (p>0.05).

Although effect sizes were minimal, previous research has noted the anti-inflammatory properties of cereal intake in their ability to prevent oxidative stress and inflammatory related diseases such as AD[9]

Images courtesy of National Institute of Health[1] - Preventing Alzheimer's Disease: What do we know?

Conclusions

This study demonstrated that MeDi adherence displayed no association with βA deposition in a cohort of healthy Australian women. WHAP is an early ageing population, thus we may not have the power to draw a directional association. With evidence that diet can impact neuropathology, further research is necessary.

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2017 International Convention of Psychological Science, Vienna, Austria: The Role of Mediterranean Diet Adherence on Cognitive Function

The Effect Of Mediterranean Diet Adherence On Full Scale IQ

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Introduction

- By 2050, it is estimated that the population of elderly people (65+ years) will double the population of children (0-14 years) for the first time in history[1]
- Global incident rates of cognitive impairment and dementia are rising
- Lack of efficacious pharmaceuticals
- Research is shifting towards modifiable lifestyle risk factors that display neuroprotective effect
- Epidemiological studies have shown that diets rich in anti-inflammatory agents may lower risk for AD and cognitive decline[1]
- Adherence to the Mediterranean Diet (MeDi) has shown promising outcomes for cognitive health[2,3,4]
- The MeDi is a traditional dietary pattern characterised by a high intake of vegetables, legumes, fruits and cereals, a moderate intake of fish and dairy and a low intake of meat and poultry
- Lack of studies investigating female populations, who are at a greater risk

Images courtesy of National Institute of Health: Preventing Alzheimer's Disease Whatdowe know?

The global increase in the ageing population has resulted in growing incident rates of cognitive impairment and dementia. In the absence of a successful cure, there have been calls for effective intervention strategies for delaying or preventing cognitive impairment and dementia.

A growing body of research has found that dietary patterns, such as the MeDi exhibit neuroprotective effects. However, there have been mixed findings regarding MeDi adherence and cognitive health, in part due to differences in the stage of decline.

Many studies have examined MeDi in impaired or diseased populations, with a subsequent paucity of findings from healthy or prodromal individuals. Furthermore, few studies investigate female only populations who are more at risk of AD and dementia [5]

Aim & Hypothesis

- To investigate the impact of MeDi adherence on Full Scale IQ in a healthy cohort of ageing Australian women in the Melbourne metropolitan area. It was hypothesized that greater MeDi adherence would be associated with an increased cognitive function

Method

- 187 healthy ageing Australian women
- Dietary Questionnaire (DQESv2) responses used to calculate MeDi adherence score[6]
- Weschler Test of Adult Reading (WTAR) used to calculate FSIQ[8,9]

Participants were 187 older women from the epidemiologically-sampled Women's Healthy Ageing Project (WHAP). Participants completed the Dietary Questionnaire for Epidemiological Studies Version 2 (DQES v2), a validated food frequency questionnaire as collated by the Cancer Council of Victoria.

The MeDi score (18 point scale) was calculated based on intake of vegetables, legumes, fruit and nuts, dairy, cereals, meat products, fish, alcohol and the monounsaturated fats to saturated fats ratio according to a literature based method[6]

Participants also completed a comprehensive neuropsychological battery of cognitive testing during assessment. The Wechsler Test of Adult Reading (WTAR), was used to calculate a full scale IQ (FSIQ). WTAR is a stable measure of intelligence used to detect premorbid differences[8,9]

Complete data was available for 187 participants. Statistical regression analysis accounted for age, education, mood, BMI, energy intake and presence of the apolipoprotein epsilon 4 (APOE-ε4) allele.

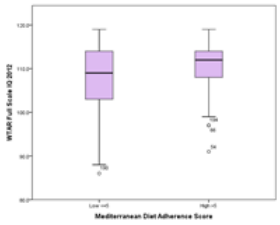
Results

Figure 1. Characteristics of the cohort included in analysis (n = 187)

	Mean ± SD (n = 187)
Age (in years)	69.9 ± 2.6
Education (in years)	12.6 ± 3.7
Energy (kJ/day)	5311.6 ± 1635.8
BMI	29.3 ± 20.4
APOE-ε4 Positive n (%)	58 (31.0%)
MeDi Adherence	5.9 ± 2.0

Characteristics of the cohort are displayed in Figure 1. MeDi adherence was significantly associated with FSIQ, accounting for all covariates ($p < 0.01$). Increasing MeDi adherence by three points led to a more than two-point increase in full-scale IQ.

Figure 2. Boxplot of WTAR Full Scale IQ by MeDi adherence. MeDi was split into two groups: Low (MeDi Score 0-6, n = 93) and High (MeDi Score 6-18, n = 94)



Conclusion

- MeDi adherence was significantly associated with FSIQ ($p>0.05$), accounting for all covariates (age, education, energy intake, BMI and APOE-ε4 presence)
- Increasing MeDi adherence by three points (on 18 point scale) was associated with a two-point increase in FSIQ
- The direction of the observed association was outside the scope of this study
- These findings provide further evidence for the positive association between MeDi adherence and cognitive function in healthy older Australian women.

Images courtesy of National Institute of Health: Preventing Alzheimer's Disease Whatdowe know?

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Acknowledgements

We would like to acknowledge the contribution of the participants and their supporters for their time and commitment for over 20 years to the University. We also thank the research assistants who assisted in data collection.

Funding for the Healthy Ageing Project (HAP) has been provided by the National Health and Medical Research Council (NHMRC Grants 547500, 1032350 & 1062133), Ramaciotti Foundation, Australian Healthy Ageing Organisation, the Brain Foundation, the Alzheimer's Association (NIA320312), Australian Menopausal Society, Bayer Healthcare, Shepherd Foundation, Scobie and Claire Mackinnon Foundation, Collier Trust Fund, J.O. & J.R. Wicking Trust, Mason Foundation and the Alzheimer's Association of Australia. The Principal Investigator of HAP (CS) is supported by the National Health and Medical Research Council and the Faculty of Medicine, Dentistry and Health Sciences at the University of Melbourne.

2018 Australasian Epidemiological Association Annual Scientific Meeting, Perth, Australia: BrainyApp: A Smartphone intervention application for lifestyle modification

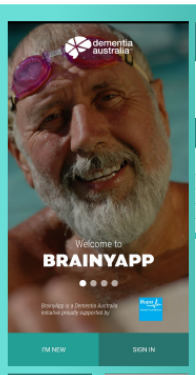
BrainyApp: A smartphone intervention application for lifestyle modification

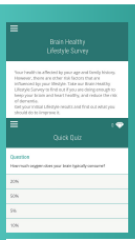
Edward Hill[1,2], Alicia M Goodwill[1,2], Alexandra Gorelik[1,2], Cassandra Szoeke[1,2]

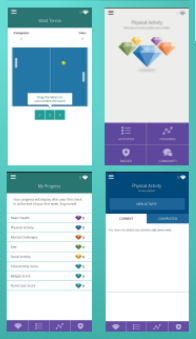
[1]University of Melbourne, Department of Medicine, Royal Melbourne Hospital, [2] School of Psychology, Australian Catholic University

In Summary:

- There are limitations in the efficacy of interventions to promote healthy lifestyle behaviour.
- The availability and accessibility of smartphones are introducing new avenues for mHealth intervention
- To guide the utility of mHealth applications as lifestyle interventions, we investigated uptake, usage and users of the **BrainyApp**
- 9580 subjects downloaded BrainyApp, 8904 (92.2%) logged in
- Out of the 7787 BrainyApp users that provided demographic information; 72.7% (n = 5663) were female, mean age was 49.7 years (SD = 17.5).
- A total of 3,693 activities were reported, with physical activities being most prevalent (78.8%) and social activities the least (2.8%).
- Uptake of healthy activities over time was predominantly seen for physical activity and in female participants.
- Multimodal interventions via mHealth display potential for promoting, improving and maintaining healthy lifestyle behaviours that promote brain health.







Results II

- Uptake of healthy activities over time was predominantly seen in female participants.
- Of those who reported a category deficit in the Lifestyle Survey, recommended uptake of healthy activities was mostly seen in the physical activity category, where 19% of users that reported a deficit engaged in an activity through the app.

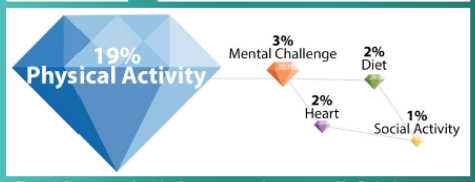


Figure 1: Percentage of uptake for survey completers, grouped by BrainyApp category

	Overall	Completed Survey	Played >1 game	Completed Quiz	p value
Female	5663 (72.7%)	5118 (70.1%)	2202 (75.7%)	1930 (73.0%)	<0.001
Male	2057 (26.4%)	2121 (29.0%)	667 (22.9%)	688 (26.0%)	
Other /not stated	67 (0.9%)	64 (0.9%)	39 (1.3%)	25 (0.9%)	
Age M (SD)	49.7 (17.5)	48.9 (17.0)	50.8 (15.4)	50.0 (16.5)	<0.001
Total	7787	7303	2908	2651	

Table 1: BrainyApp User Demographics by level of application engagement

Introduction

- Despite considerable research suggesting modifiable lifestyle factors contribute to the incidence and prevalence of cognitive decline, there are limitations in the efficacy of interventions to promote healthy lifestyle behaviour.

Aim

- Our aim was to investigate the uptake, usage and user characteristics of the BrainyApp in order to guide the utility of future mobile phone applications as lifestyle interventions.

Method

- Dementia Australia, in partnership with Bupa Health Foundation, developed the BrainyApp. Participants from around Australia downloaded the BrainyApp mobile phone application on iOS or Android device. Stratified descriptive statistics were produced for: Demographics, Brain Health Survey, Brain Game, Quick Quiz, Activities.

Results I

- Between February 2016 and March 2017, 9580 subjects downloaded BrainyApp.
- Out of the 7787 BrainyApp users that provided demographic information; 72.7% (n = 5663) were female, mean age was 49.7 years (SD = 17.5).
- BrainyApp users showed significantly higher compliance to Australian guidelines on smoking and consumption of fruits and vegetables
- Females made up the majority of users in each age category and an increasing number of users were observed as age increased for both males and females
- Female participants made up the majority of users who completed the Brain Health Survey (70.1%), played at least one Brain Game (75.7%) and participated in the Quick Quiz (73.0%)
- A total of 2914 BrainyApp users played the "Word Tennis" brain training game at least once.
- A total of 3,693 activities were reported, with physical activities being most prevalent (78.8%) and social activities the least (2.8%).

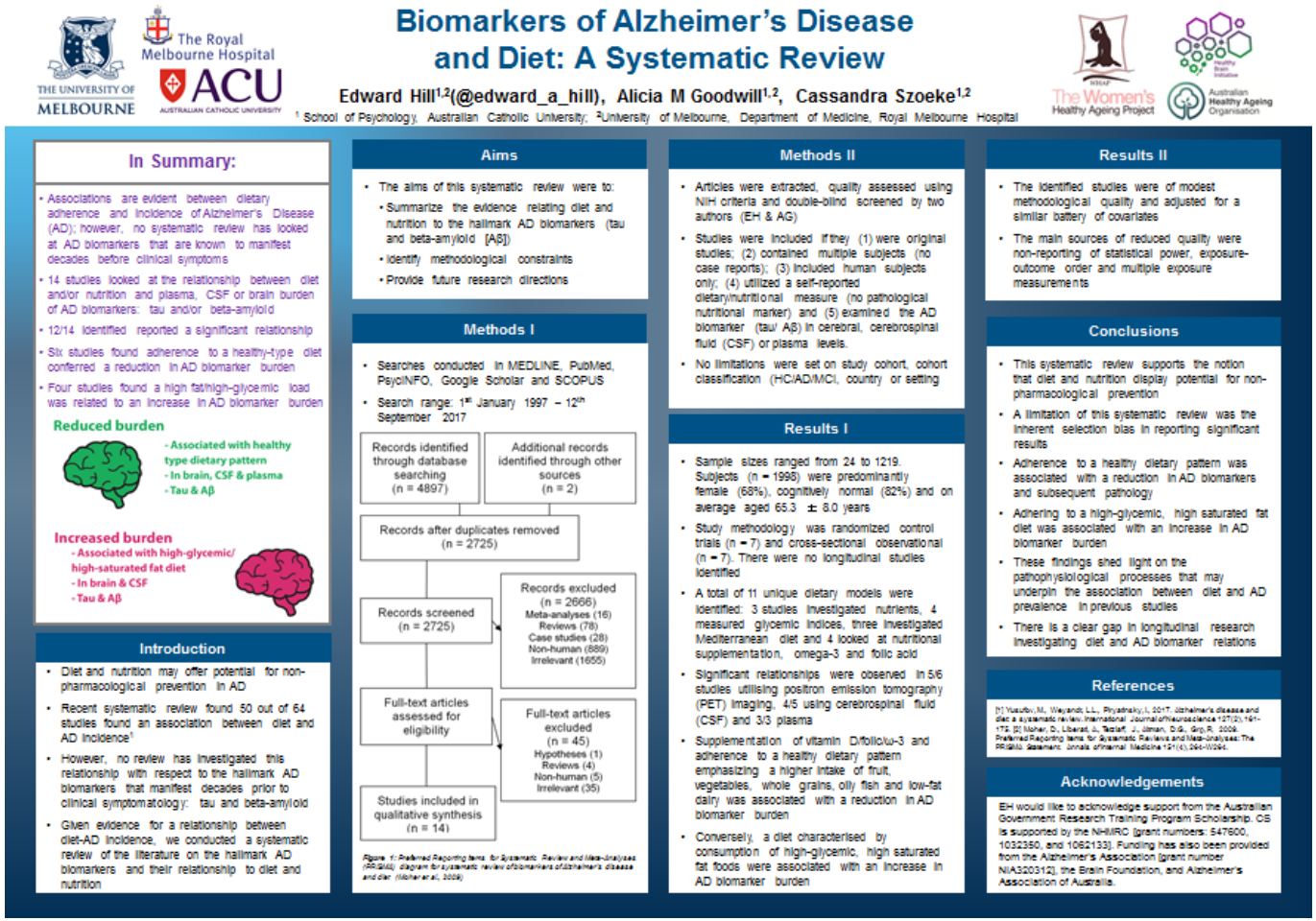
Conclusions

- BrainyApp received a large amount of downloads and usage from a wide variety of age and gender groups around Australia.
- BrainyApp displayed a strong focus on community engagement, with many positive responses appearing on the BrainyApp wall within the application.
- Uptake of activities was seen predominantly in physical activity
- Given the current research supports the notion that modifiable lifestyle risk factors contribute to risk of cognitive decline, multimodal interventions via mHealth display potential for promoting, improving and maintaining healthy lifestyle behaviours that promote brain health.

Acknowledgements

EH would like to acknowledge support from the Australian Government Research Training Program Scholarship. CS is supported by the NHMRC [grant numbers: 547600, 1032350, and 1062133]. Funding has also been provided from the Alzheimer's Association [grant number NIA320312], the Brain Foundation, and Dementia Australia. Images are screenshots from within the BrainyApp.

2018 Alzheimer's Association International Conference, Chicago, USA: Biomarkers of Alzheimer's disease and diet: A systematic review



2019 Research Bazaar, University of Melbourne

Edward Hill

PhD, Neuroscience

@edward_a_hill

@ddhll - https://ddhll.github.io/

My Research: Investigating the preventative potential of diet in the pre-clinical stages of neuropathology in Alzheimer's disease (AD). AD is a devastating disease of global epidemic proportions. With no current pharmacological treatment, my research is investigating potential modifiable risk factors, such as diet, for therapeutic efficacy.

My digital toolbox:

- SPSS/R/STATA for analysis
- Photoshop/Plot.ly for dataviz

I've got my eyes on: BigQuery (I would like to learn how to query big data sets using online APIs)

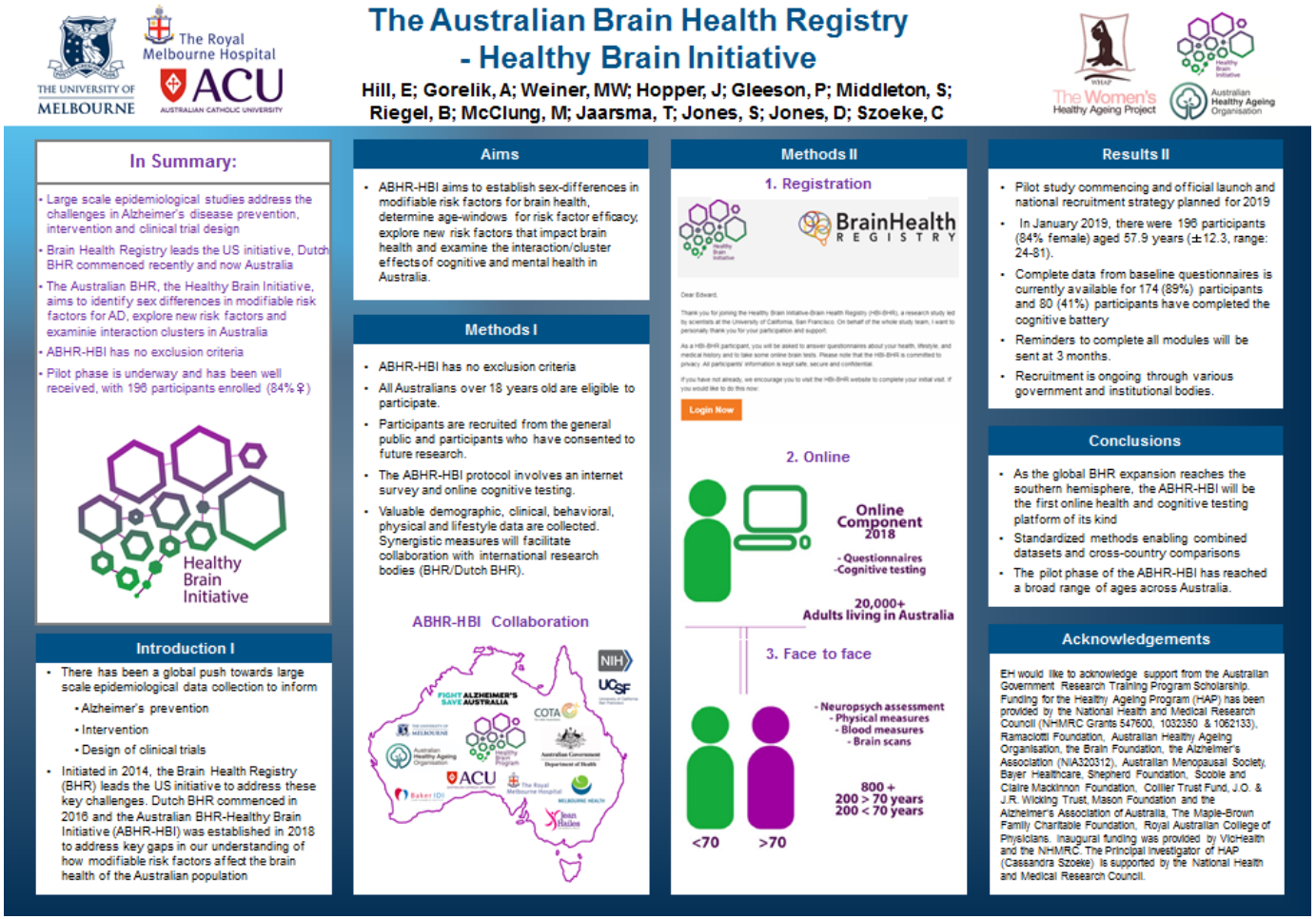
My favourite tool:

STATA

STATA was the first command line data analysis I used and for that reason it has a special place in my heart. I also use two vertical monitors and STATA's UI is perfect for my set up.

Research SOS: My research regards the computation of missing data. In epidemiological research, missing data is not randomly distributed. Often health complications and contra-indications impart a selective bias into the cohort that undergoes analysis. In my research I have often omitted those with any missing data in the independent/dependent variables or covariates, yet this is also omitting their other variables. To maximise the collated data I would like to know how to tackle missing data in epidemiological research.

2019 Alzheimer's Association International Conference, The Australian Brain Health Registry-Healthy Brain Initiative



APPENDIX 6: PLATFORM PRESENTATIONS

A number of platform presentations were presented throughout my PhD candidature. Presented are the title slides. Full presentation slides are available upon request.

2017 Alzheimer's Association International Conference, London, UK: Family History of Dementia Impacts Dietary Inflammatory Index Scores in Healthy Ageing Australian Women – a Case of Reverse Causality?

Family History of Dementia Impacts Dietary Inflammatory Index Scores in Healthy Ageing Australian Women – A Case of Reverse Causality?

Edward Hill, Allison Hodge, Nitin Shivappa, Professor Cassandra Szoeke
edward.hill@myacu.edu.au
@edward_a_hill

Alzheimer's Association International Conference
Nutrition, Metabolism and Dementia PIA
London, England
Saturday July 15, 2017

The authors note no conflict of interest

2018 Alzheimer's Disease International, Chicago, USA: Dietary patterns and beta-amyloid deposition in ageing Australian women

Dietary Patterns and Beta-Amyloid Deposition in Ageing Australian Women

Hill, Edward[a, b] ; Clifton, Peter [c]; Goodwill, Alicia[a,b]; Dennerstein, Lorraine[d]; Campbell, Stephen[e]; Szoeke, Cassandra[a, b]

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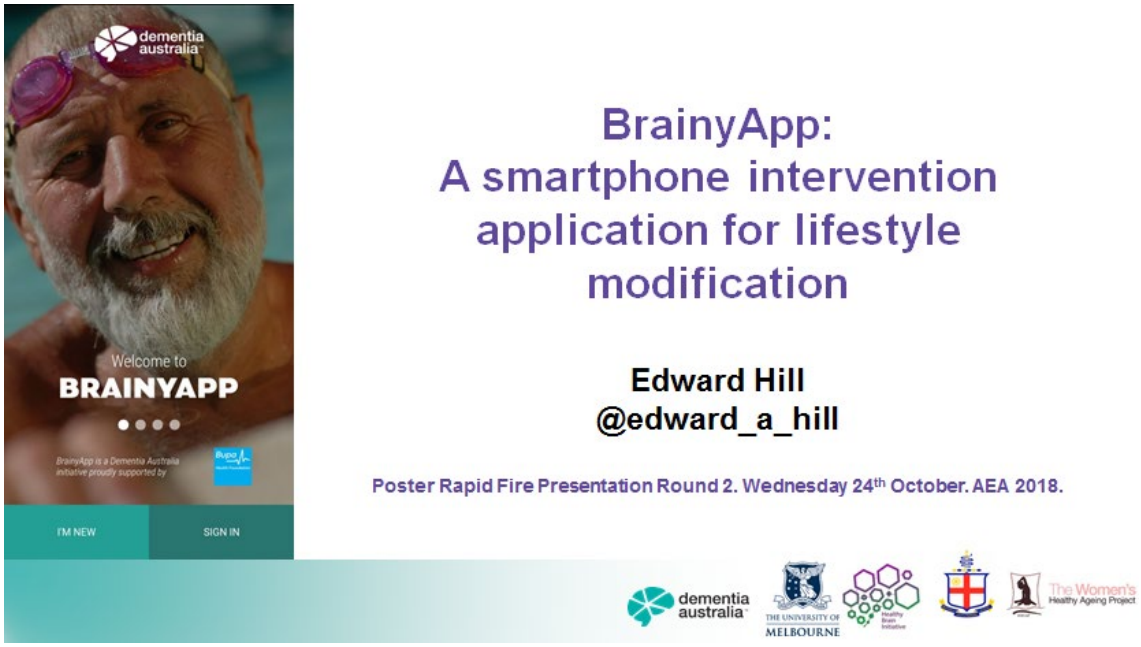
[b] Centre for Medical Research, Department of Medicine-Royal Melbourne Hospital, University of Melbourne, Parkville, Victoria 3050, Australia
[c] School of Pharmacy and Medical Sciences, University of South Australia, 101 Currie St, Adelaide, South Australia 5001, Australia
[d] Department of Psychiatry, University of Melbourne, Parkville, Victoria 3050, Australia
[e] Melbourne Health, Melbourne, Australia

@edward_a_hill

Alzheimer's Disease International Conference
196c, McCormick Place West, Chicago, USA
Saturday July 28, 2018: 2:00pm – 3:30pm

2018 Australasian Epidemiological Association Annual Scientific Meeting, Perth, Australia: BrainyApp: A Smartphone intervention application for lifestyle modification



APPENDIX 7: PUBLICATIONS

Hill E, Goodwill AM, Gorelik A, Szoeke C. Diet and biomarkers of Alzheimer's disease: a systematic review and meta-analysis. Neurobiology of Aging. 2019; April: 45-52.

Neurobiology of Aging 76 (2019) 45–52

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Review

Diet and biomarkers of Alzheimer's disease: a systematic review and meta-analysis

Edward Hill, M. Alicia Goodwill, Alexandra Gorelik, Cassandra Szoeke*

Faculty of Health Sciences, School of Psychology, Australian Catholic University, Melbourne, Australia
Institute for Health & Ageing, Australian Catholic University, Melbourne, Australia
Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, Melbourne, Australia

ARTICLE INFO

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Keywords:
Alzheimer's disease
Biomarkers
Tau
Beta-amyloid
Diet
Nutrition

ABSTRACT

Alzheimer's disease (AD) risk increases with age and lacks efficacious pharmacological options. Summaries of the existing evidence reveal an association between Mediterranean-style diet adherence and reduced AD incidence; however, no review has investigated this relationship with respect to the hallmark AD biomarkers (tau and beta-amyloid) that manifest decades before clinical symptomatology. MEDLINE, PubMed, PsycINFO, Google Scholar, and SCOPUS databases were systematically searched to identify peer-reviewed articles investigating diet and AD biomarkers in the last 2 decades. Two thousand seven hundred twenty-six records were extracted, quality assessed, and double-blind screened by 2 authors. Fifteen studies met the inclusion criteria and 13 studies found a significant relationship. Of these, 4 studies found a high-glycemic load was related to an increase in AD biomarker burden; 6 found adherence to a Mediterranean or "AD-protective" dietary pattern conferred a reduction in AD biomarker burden. Meta-analysis revealed a small but significant effect of diet on AD biomarkers ($\beta = 0.11$ [95% CI 0.04–0.17], $p = 0.002$). This systematic review supports the notion that diet and nutrition display potential for nonpharmacological AD prevention.

1. Introduction

Dementia incidence exponentially increases with age, doubling approximately every 5 years (Jorm and Jolley, 1998). Alzheimer's disease (AD) is the leading cause of dementia, accounting for 60%–70% of all cases (Fratiglioni and Rocca, 2001) and is a global public health priority. The *Alzheimer's Disease International* World Alzheimer Report 2015 estimated 46.8 million people living with dementia worldwide, and this number is expected to reach 131.5 million by 2050 (Prince et al., 2015). Current pharmacological approaches, including cholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists, are focused on treating AD symptomatology and are lacking therapeutic efficacy. Clinical trials that focus on reducing the pathological burden of AD biomarkers are failing (Mehta et al., 2017). Given the resistance to treatment, a growing body of research is now focusing on the preventative potential of modifiable risk factors in the AD pathology before the emergence of clinical symptoms.

Nonmodifiable risk factors for AD include family history, aging, head injury, and carrying the epsilon 4 allele of the apolipoprotein E gene (APOE-ε4). The *2017 Lancet Commission on Dementia Prevention, Intervention and Care* found that approximately 35% of dementia is attributable to potentially modifiable lifestyle risk factors (Livingston et al., 2017). Epidemiological evidence indicates that up to 3 million AD cases worldwide could be prevented with a 10%–25% reduction in known modifiable midlife risk factors (Barnes and Yaffe, 2011).

Diet and nutrition may offer potential for nonpharmacological prevention in AD. Epidemiological findings are consistent in showing that high adherence to dietary patterns characterized by high intake of fruit, vegetables, cereals and legumes and low intake of meat, high-fat dairies, and sweets are consistently associated with a lower risk of AD (Feart et al., 2009; Gardener et al., 2012; Gu and Scarmeas, 2011; Scarmeas et al., 2006, 2007). Prospective studies have also suggested that low to moderate alcohol consumption (Piazza-Gardner et al., 2013), lower carbohydrate consumption (Seneff et al., 2011), and increased vitamin intake (Engelhart et al., 2002) are associated with a decreased risk for AD. In Australia, increased adherence to a Mediterranean diet (MeDi) has been associated with changes in mini-mental state examination over 18 months (Gardener et al., 2012) and a reduction in risk for

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Hill E, Hodge A, Clifton P, Shivappa N, Hebert JR, Dennerstein L, Campbell S, Szoeke C. Longitudinal nutritional changes in aging Australian women. Asia Pac J Clin Nutr. 2019;28:139.

Asia Pac J Clin Nutr 2019;28(1):139-149

Original Article

Longitudinal nutritional changes in aging Australian women

Edward Hill BSc[1,2], Allison Hodge PhD[3,4], Peter Clifton FRACP[5], Nitin Shivappa PhD[6,7,8], James R Hebert ScD[6,7,8], Lorraine Dennerstein AO[9], Stephen Campbell FRACP[10], Cassandra Szoeke GAICD[1,2]

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[2]*Institute for Health & Ageing, Melbourne, Victoria, Australia*
[3]*Cancer Epidemiology and Intelligence Division, Cancer Council of Victoria, Melbourne, Victoria, Australia*
[4]*Centre for Epidemiology and Biostatistics, University of Melbourne, Australia*
[5]*School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia*
[6]*South Carolina Statewide Cancer Prevention and Control Program, University of South Carolina, Columbia, USA*
[7]*Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, USA*
[8]*Connecting Health Innovations LLC, Columbia, USA*
[9]*Department of Psychiatry, University of Melbourne, Victoria, Australia*
[10]*Melbourne Health, Melbourne, Australia*

Background and Objectives: The importance of diet for the maintenance of health during aging is attracting a growing body of research interest. Given dietary intakes, along with BMI, are substantial contributors to disease burden, this study aimed to investigate prospective changes in dietary patterns and nutrient intakes in a sample of mid to late-life women over 14 years. **Methods and Study Design:** Participants were from the Women's Healthy Ageing Project (WHAP); a longitudinal cohort of Australian-born women within the Melbourne metropolitan area. 173 participants were included in this analysis, their mean age in 1998 was 55 years (range 51-62) and in 2012 was 70 years (range 66-76). Diet was assessed using the Dietary Questionnaire for Epidemiological Studies Version 2 in 1998 and 2012. Nutritional intakes, Dietary Inflammatory Index (DII®) scores, Mediterranean Diet (MD) scores, sociodemographic and physical measures were calculated for all participants at both time points. **Results:** Energy intake was found to significantly decrease over time ($p<0.005$). Energy-adjusted (i.e., energy density) total fat, saturated fat, monounsaturated fat and cholesterol intakes increased over time (all $p<0.002$), while energy-adjusted and absolute carbohydrate intake decreased ($p<0.002$). Adherence to the MD decreased over time ($p<0.001$) whilst DII scores increased slightly over time, although this result was not significant. **Conclusions:** This study shows significant changes in the intake of energy and several nutrients in a cohort of aging Australian women in the Melbourne metropolitan area over a period of 14 years. Between 1998 and 2012, changes in indices reflecting overall diet were consistently in the direction of a poorer diet.

Key Words: epidemiology, nutrition, Mediterranean diet, dietary inflammatory index, prospective studies

INTRODUCTION

With global populations surviving longer, research is shifting focus toward the promotion of healthy aging. A growing body of research acknowledges the importance of diet for the maintenance of health during aging.[1] Because there is a natural decrease in caloric intake with aging,[2,3] the investigation of nutritional sufficiency in aging adults is particularly important in order to maximize quality of life and the independence of individuals in the community. Over the last few decades there has been a great change in the cooking and eating habits of the Australian population. Greater consumption of energy-dense foods and larger portion sizes have resulted in excessive energy intake, leading to overweight and obesity.[4] A comparative investigation into the 1995 and 2011-2012

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Manuscript received 13 September 2018. Initial review completed 14 October 2018. Revision accepted 11 November 2018.
doi: 10.6133/apjcn.201903_28(1).0019

Hill E, Clifton P, Goodwill AM, Dennerstein L, Campbell S, Szoeke CJAs, Research DT, Interventions C. Dietary patterns and β-amyloid deposition in aging Australian women. 2018;4:535-41.

ELSEVIER

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Alzheimer's & Dementia: Translational Research & Clinical Interventions 4 (2018) 535-541

Alzheimer's & Dementia

Featured Article

Dietary patterns and β-amyloid deposition in aging Australian women

Edward Hill[a,b,c], Peter Clifton[d], Alicia M. Goodwill[a,b,c], Lorraine Dennerstein[e], Stephen Campbell[f], Cassandra Szoeke[a,b,c,*]

[a]Department of Medicine (Royal Melbourne Hospital), University of Melbourne, Parkville, Victoria, Australia
[b]School of Psychology, Australian Catholic University, Melbourne, Victoria, Australia
[c]Institute for Health and Ageing, Australian Catholic University, Melbourne, Victoria, Australia
[d]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia
[e]Department of Psychiatry, University of Melbourne, Parkville, Victoria, Australia
[f]Melbourne Health, Melbourne, Australia

Abstract

Introduction: Evidence indicates that associations between diet and Alzheimer's disease may occur through biomarker pathways such as amyloid-β (Aβ); however, few studies have investigated dietary/Aβ relationships, and no study has investigated this relationship in women.
Methods: Dietary patterns were extrapolated for 115 participants from the Women's Health Aging Project. Aβ deposition was measured via *in vivo* F-18 florbetaben positron emission tomography scanning.
Results: Participants were, on average, aged 70 years (±2.63 SD), had 13 years of education (±3.57 SD), a BMI of 28 kg/m^2 (±5.46 SD), and a daily energy intake of 5161 kJ (±1679.03 SD). Four dietary patterns were identified: high fat, Mediterranean, junk food, and low fat. Adherence to the junk food diet was a significant predictor of Aβ deposition (β = .10, P = .03).
Discussion: This study highlights the potential of diet to influence neurodegenerative disease and as a potential modifiable lifestyle risk factor for Alzheimer's disease.

Keywords: Biomarkers; Alzheimer's disease; Neuropathology; β-amyloid protein; Diet; Nutrition; Dietary pattern; Factor analysis; Women

1. Introduction

Diet may play a substantial role in the Alzheimer's disease (AD) symptomatology and offer great potential for non-pharmacological prevention. Epidemiological evidence has suggested increased adherence to a Mediterranean diet [1], low glycemic index [2,3], and higher consumption of omega-3 polyunsaturated fatty acids [4] were associated with a decrease in AD biomarker burden. Systematic review found 50 out of 64 studies revealed an association between diet and AD incidence [5]; however, only one study has used *a priori* analysis to analyze dietary associations with the hallmark cerebral protein implicated in AD, β-amyloid (Aβ). In this study, dietary pattern analysis identified a pattern characterized by a higher intake of fresh fruit, vegetables, whole grains, fish, and low-fat dairies, and a lower intake of sweets, fried potatoes, processed meat, and butter was negatively associated with *in vivo* cerebral Aβ [6].

Furthermore, male and mixed cohort studies predominate the research, and to date, no study has investigated this relationship specifically in women. Women are more likely than men to develop AD [7], have a higher penetrance for the apolipoprotein ε-4 (APOE-ε4) allele [8], and are more likely to progress from mild cognitive impairment to AD [8]. Impacts of higher male mortality, vascular risk factors, and the postmenopausal loss of estrogenic neuroprotection suggest females are 1.5 times more likely to develop AD than men [9]. Given sex differences in AD risk, research is needed for those at greater risk of disease.

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https://doi.org/10.1016/j.trci.2018.09.007

APPENDIX 8: STATEMENT OF CONTRIBUTIONS

Statement of Contribution to Jointly Published Work – Chapter 2

Hill E, Goodwill AM, Gorelik A, Szoeke C. Diet and biomarkers of Alzheimer's disease: a systematic review and meta-analysis. Neurobiology of Aging. 2019; April: 45-52.

Edward Hill	Study conception and design Screening of articles Preparation of draft manuscripts Approval of final version of the manuscript for publication and submission of the article to the journal for publication
Alicia Goodwill	Provision of advice on conception of study, data collection and data analysis Screening of articles Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication
Alexandra Gorelik	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of syntax for quantitative analysis Approval of final version of the manuscript for publication
Cassandra Szoeke	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication

Signed: 

Name: Edward Hill

Date: 23rd January 2020

Statement of Contribution to Jointly Published Work – Chapter 4

Hill E, Hodge A, Clifton P, Shivappa N, Hebert JR, Dennerstein L, Campbell S, Szoeke C. Longitudinal nutritional changes in aging Australian women. Asia Pacific Journal of Clinical Nutrition. 2019;28:139.

Edward Hill	Study conception and design Preparation of draft manuscripts Statistical analysis Approval of final version of the manuscript for publication and submission of the article to the journal for publication
Allison Hodge	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of assistance with statistical analysis Approval of final version of the manuscript for publication
Nitin Shivappa	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of Dietary Inflammatory Index scores Approval of final version of the manuscript for publication
James Hebert	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of Dietary Inflammatory Index scores Approval of final version of the manuscript for publication
Lorraine Dennerstein	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication

Stephen Campbell	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication
Cassandra Szoeke	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication

Signed: 

Name: Edward Hill

Date: 23rd January 2020

Statement of Contribution to Jointly Published Work – Chapter 6

Hill E, Clifton P, Goodwill AM, Dennerstein L, Campbell S, Szoeke C. Neurobiology of Aging. Dietary patterns and β-amyloid deposition in aging Australian women. 2018;4:535-41.

Edward Hill	Study conception and design Preparation of draft manuscripts Statistical analysis Approval of final version of the manuscript for publication and submission of the article to the journal for publication
Peter Clifton	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of assistance with statistical analysis Approval of final version of the manuscript for publication
Alicia Goodwill	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Provision of assistance with statistical analysis Approval of final version of the manuscript for publication
Lorraine Dennerstein	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication
Stephen Campbell	Provision of advice on conception of study, data collection and data analysis Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication
Cassandra Szoeke	Provision of advice on conception of study, data collection and data analysis

	Provision of revisions to draft manuscripts Approval of final version of the manuscript for publication

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